CORYNEBACTERIUM GLUTAMICUM GENES ENCODING PROTEINS INVOLVED IN GENETIC STABILITY, GENE EXPRESSION, AND PROTEIN SECRETION AND FOLDING

Related Applications

This application claims priority to prior filed U.S. Provisional Patent Application Serial No. 60/141031, filed June 25, 1999, U.S. Provisional Patent Application Serial No. 60/143752, filed July 14, 1999, and U.S. Provisional Patent Application Serial No. 60/151671, filed August 8, 1999. This application also claims priority to prior filed German Patent Application No. 19931412.8, filed July 8, 1999, and German Patent Application No. 19932928.1, filed July 14, 1999. The entire contents of all of the aforementioned applications are expressly incorporated herein by this reference.

09650290 .060900

Background of the Invention

Certain products and by-products of naturally-occurring metabolic processes in cells have utility in a wide array of industries, including the food, feed, cosmetics, and pharmaceutical industries. These molecules, collectively termed 'fine chemicals', include organic acids, both proteinogenic and non-proteinogenic amino acids, nucleotides and nucleosides, lipids and fatty acids, diols, carbohydrates, aromatic compounds, vitamins and cofactors, and enzymes. Their production is most conveniently performed through the large-scale culture of bacteria developed to produce and secrete large quantities of one or more desired molecules. One particularly useful organism for this purpose is *Corynebacterium glutamicum*, a gram positive, nonpathogenic bacterium. Through strain selection, a number of mutant strains have been developed which produce an array of desirable compounds. However, selection of strains improved for the production of a particular molecule is a time-consuming and difficult process.

Summary of the Invention

The invention provides novel bacterial nucleic acid molecules which have a variety of uses. These uses include the identification of microorganisms which can be used to produce fine chemicals, the modulation of fine chemical production in *C. glutamicum* or related bacteria, the typing or identification of *C. glutamicum* or related bacteria, as reference points for mapping the *C. glutamicum* genome, and as markers for transformation. These novel nucleic acid molecules encode proteins, referred to herein as stability, gene expression, or protein secretion/folding (SES) proteins.

C. glutamicum is a gram positive, aerobic bacterium which is commonly used in industry for the large-scale production of a variety of fine chemicals, and also for the degradation of hydrocarbons (such as in petroleum spills) and for the oxidation of terpenoids. The SES nucleic acid molecules of the invention, therefore, can be used to identify microorganisms which can be used to produce fine chemicals, *e.g.*, by fermentation processes. Modulation of the expression of the SES nucleic acids of the invention, or modification of the sequence of the SES nucleic acid molecules of the invention, can be used to modulate the production of one or more fine chemicals from a microorganism (*e.g.*, to improve the yield or production of one or more fine chemicals from a Corynebacterium or Brevibacterium species).

The SES nucleic acids of the invention may also be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof, or to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present. Although *Corynebacterium glutamicum* itself is nonpathogenic, it is related to species pathogenic in humans, such as *Corynebacterium diphtheriae* (the causative agent of diphtheria); the detection of such organisms is of significant clinical relevance.

The SES nucleic acid molecules of the invention may also serve as reference points for mapping of the *C. glutamicum* genome, or of genomes of related organisms. Similarly, these molecules, or variants or portions thereof, may serve as markers for genetically engineered Corynebacterium or Brevibacterium species.

*e.g.e.g.*The SES proteins encoded by the novel nucleic acid molecules of the invention are capable of, for example, performing a function involved in the repair or recombination of DNA, transposition of genetic material, expression of genes (*i.e.*, involved in transcription or translation), protein folding, or protein secretion in *Corynebacterium glutamicum*. Given the availability of cloning vectors for use in *Corynebacterium glutamicum*, such as those disclosed in Sinskey et al., U.S. Patent No. 4,649,119, and techniques for genetic manipulation of *C. glutamicum* and the related *Brevibacterium* species (*e.g.*, *lactofermentum*) (Yoshihama et al, *J. Bacteriol.* 162: 591-597 (1985); Katsumata et al., *J. Bacteriol.* 159: 306-311 (1984); and Santamaria et al., *J. Gen. Microbiol.* 130: 2237-2246 (1984)), the nucleic acid molecules of the invention may be utilized in the genetic engineering of this organism to make it a better or more efficient producer of one or more fine chemicals. This improved production or

efficiency of production of a fine chemical may be due to a direct effect of manipulation of a gene of the invention, or it may be due to an indirect effect of such manipulation.

There are a number of mechanisms by which the alteration of an SES protein of the invention may directly affect the yield, production, and/or efficiency of production of a fine chemical from a *C. glutamicum* strain incorporating such an altered protein. For example, modulation of proteins involved directly in transcription or translation (*e.g.*, polymerases or ribosomes) such that they are increased in number or in activity should increase global cellular transcription or translation (or rates of these processes). This increased cellular gene expression should include those proteins involved in fine chemical biosynthesis, so an increase in yield, production, or efficiency of production of one or more desired compounds may occur. Modifications to the transcriptional/ translational protein machinery of *C. glutamicum* such that the regulation of these proteins is altered may also permit increased expression of genes involved in the production of fine chemicals. Modulation of the activity or number of proteins involved in polypeptide folding may permit an increase in the overall production of correctly folded molecules in the cell, thereby increasing the possibility that desired proteins (*e.g.*, fine chemical biosynthetic proteins) are able to function properly. Further, by mutating proteins involved in secretion from *C. glutamicum* such that they are increased in number or activity, it may be possible to increase the secretion of a fine chemical (*e.g.*, an enzyme) from cells in fermentor culture, where it may be readily recovered.

Genetic modification of the SES molecules of the invention may also result in indirect modulation of production of one or more fine chemicals. For example, by increasing the number or activity of a DNA repair or recombination protein of the invention, one may increase the ability of the cell to detect and repair DNA damage. This should effectively increase the ability of the cell to maintain a mutated gene within its genome, thereby increasing the likelihood that a transgene engineered *into C. glutamicum* (*e.g.*, encoding a protein which will increase biosynthesis of a fine chemical) will not be lost during culture of the microorganism. Conversely, by decreasing the number or activity of one or more DNA repair or recombination proteins, it may be possible to increase the genetic instability of the organism. Such manipulations should improve the ability of the organism to be modified by mutagenesis without the introduced mutation being corrected. The same holds true for proteins involved in transposition or rearrangement of genetic elements in *C. glutamicum* (*e.g.*, transposons). By mutagenizing these proteins such that they are either increased or decreased in number or activity, it is possible to simultaneously increase or decrease the genetic stability of the microorganism. This has a profound impact on the ability of any other mutation to be introduced into *C. glutamicum*, and on the ability of introduced

mutations to be retained. Transposons also offer a convenient mechanism by which mutagenesis of *C. glutamicum* may be performed; duplication of desired genes (*e.g.*, fine chemical biosynthetic genes) is readily accomplished by transposon mutagenesis, as is disruption of undesired genes (*e.g.*, genes encoding proteins involved in degradation of desired fine chemicals).

By modulating one or more proteins (*e.g.* sigma factors) involved in the regulation of transcription or translation in response to particular environmental conditions, it may be possible to prevent the cell from slowing or stopping protein synthesis under unfavorable environmental conditions, such as those found in large-scale fermentor culture. This should lead to increased gene expression, which in turn may permit increased biosynthesis of desired fine chemicals under such conditions. Mutagenesis of proteins involved in protein secretion systems may result in modulated secretion rates. Many such secreted proteins have functions critical for cell viability (*e.g.*, cell surface proteases or receptors). An alteration of a secretory pathway such that these proteins are more readily transported to their extracellular location may improve the overall viability of the cell, and thus result in greater numbers of *C. glutamicum* cells capable of producing fine chemicals during large-scale culture. Further, the secretion apparatus (*e.g.*, the sec system) is also known to be involved in the insertion of integral membrane proteins (*e.g.*, pores, channels, or transporters) into the membrane. Thus, the modulation of activity of proteins involved in protein secretion from *C. glutamicum* may affect the ability of the cell to excrete waste products or to import necessary metabolites. If the activity of these secretory proteins is increased, then the ability of the cell to produce fine chemicals may be similarly increased. If the activity of these secretory proteins is decreased, then there may be insufficient nutrients available to support overproduction of desired compounds, or waste products may interfere with such biosynthesis.

The invention provides novel nucleic acid molecules which encode proteins, referred to herein as SES proteins, which are capable of, for example, participating in the repair or recombination of DNA, transposition of genetic material, expression of genes (*i.e.*, the processes of transcription or translation), protein folding, or protein secretion in *Corynebacterium glutamicum*. Nucleic acid molecules encoding an SES protein are referred to herein as SES nucleic acid molecules. In a preferred embodiment, an SES protein participates in improving or decreasing genetic stability in *C. glutamicum*, in the expression of genes (*i.e.*, in transcription or translation) or protein folding in this organism, or in protein secretion from *C. glutamicum*. Examples of such proteins include those encoded by the genes set forth in Table 1.

Accordingly, one aspect of the invention pertains to isolated nucleic acid molecules (*e.g.*, cDNAs, DNAs, or RNAs) comprising a nucleotide sequence encoding an SES protein or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection or amplification of SES-encoding nucleic acid (*e.g.*, DNA or mRNA). In particularly preferred embodiments, the isolated nucleic acid molecule comprises one of the nucleotide sequences set forth in Appendix A or the coding region or a complement thereof of one of these nucleotide sequences. In other particularly preferred embodiments, the isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes to or is at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80% or 90%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence set forth in Appendix A, or a portion thereof. In other preferred embodiments, the isolated nucleic acid molecule encodes one of the amino acid sequences set forth in Appendix B. The preferred SES proteins of the present invention also preferably possess at least one of the SES activities described herein.

In another embodiment, the isolated nucleic acid molecule encodes a protein or portion thereof wherein the protein or portion thereof includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B, *e.g.*, sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains an SES activity. Preferably, the protein or portion thereof encoded by the nucleic acid molecule maintains the ability to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. In one embodiment, the protein encoded by the nucleic acid molecule is at least about 50%, preferably at least about 60%, and more preferably at least about 70%, 80%, or 90% and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an amino acid sequence of Appendix B (*e.g.*, an entire amino acid sequence selected from those sequences set forth in Appendix B). In another preferred embodiment, the protein is a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of Appendix B (encoded by an open reading frame shown in Appendix A).

In another preferred embodiment, the isolated nucleic acid molecule is derived from *C. glutamicum* and encodes a protein (*e.g.*, an SES fusion protein) which includes a biologically active domain which is at least about 50% or more homologous to one of the amino acid sequences of Appendix B and is able to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*,

the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or has one or more of the activities set forth in Table 1, and which also includes heterologous nucleic acid sequences encoding a heterologous polypeptide or regulatory regions.

In another embodiment, the isolated nucleic acid molecule is at least 15 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of Appendix A. Preferably, the isolated nucleic acid molecule corresponds to a naturally-occurring nucleic acid molecule. More preferably, the isolated nucleic acid encodes a naturally-occurring *C. glutamicum* SES protein, or a biologically active portion thereof.

Another aspect of the invention pertains to vectors, *e.g.*, recombinant expression vectors, containing the nucleic acid molecules of the invention, and host cells into which such vectors have been introduced. In one embodiment, such a host cell is used to produce an SES protein by culturing the host cell in a suitable medium. The SES protein can be then isolated from the medium or the host cell.

Yet another aspect of the invention pertains to a genetically altered microorganism in which an SES gene has been introduced or altered. In one embodiment, the genome of the microorganism has been altered by introduction of a nucleic acid molecule of the invention encoding wild-type or mutated SES sequence as a transgene. In another embodiment, an endogenous SES gene within the genome of the microorganism has been altered, *e.g.*, functionally disrupted, by homologous recombination with an altered SES gene. In another embodiment, an endogenous or introduced SES gene in a microorganism has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional SES protein. In still another embodiment, one or more of the regulatory regions (*e.g.*, a promoter, repressor, or inducer) of an SES gene in a microorganism has been altered (*e.g.*, by deletion, truncation, inversion, or point mutation) such that the expression of the SES gene is modulated. In a preferred embodiment, the microorganism belongs to the genus *Corynebacterium* or *Brevibacterium*, with *Corynebacterium glutamicum* being particularly preferred. In a preferred embodiment, the microorganism is also utilized for the production of a desired compound, such as an amino acid, with lysine being particularly preferred.

In another aspect, the invention provides a method of identifying the presence or activity of *Cornyebacterium diphtheriae* in a subject. This method includes detection of one or more of the nucleic acid or amino acid sequences of the invention (*e.g.*, the sequences set forth in Appendix A or Appendix B) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject.

Still another aspect of the invention pertains to an isolated SES protein or a portion, *e.g.*, a biologically active portion, thereof. In a preferred embodiment, the isolated SES protein or portion thereof can participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. In another preferred embodiment, the isolated SES protein or portion thereof is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*.

The invention also provides an isolated preparation of an SES protein. In preferred embodiments, the SES protein comprises an amino acid sequence of Appendix B. In another preferred embodiment, the invention pertains to an isolated full length protein which is substantially homologous to an entire amino acid sequence of Appendix B (encoded by an open reading frame set forth in Appendix A). In yet another embodiment, the protein is at least about 50%, preferably at least about 60%, and more preferably at least about 70%, 80%, or 90%, and most preferably at least about 95%, 96%, 97%, 98%, or 99% or more homologous to an entire amino acid sequence of Appendix B. In other embodiments, the isolated SES protein comprises an amino acid sequence which is at least about 50% or more homologous to one of the amino acid sequences of Appendix B and is able to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or has one or more of the activities set forth in Table 1.

Alternatively, the isolated SES protein can comprise an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, *e.g.*, hybridizes under stringent conditions, or is at least about 50%, preferably at least about 60%, more preferably at least about 70%, 80%, or 90%, and even more preferably at least about 95%, 96%, 97%, 98,%, or 99% or more homologous, to a nucleotide sequence of Appendix B. It is also preferred that the preferred forms of SES proteins also have one or more of the SES bioactivities described herein.

The SES polypeptide, or a biologically active portion thereof, can be operatively linked to a non-SES polypeptide to form a fusion protein. In preferred embodiments, this fusion protein has an activity which differs from that of the SES protein alone. In other preferred embodiments, this fusion protein participates in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*,

the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. In particularly preferred embodiments, integration of this fusion protein into a host cell modulates production of a desired compound from the cell.

In another aspect, the invention provides methods for screening molecules which modulate the activity of an SES protein, either by interacting with the protein itself or a substrate or binding partner of the SES protein, or by modulating the transcription or translation of an SES nucleic acid molecule of the invention.

Another aspect of the invention pertains to a method for producing a fine chemical. This method involves the culturing of a cell containing a vector directing the expression of an SES nucleic acid molecule of the invention, such that a fine chemical is produced. In a preferred embodiment, this method further includes the step of obtaining a cell containing such a vector, in which a cell is transfected with a vector directing the expression of an SES nucleic acid. In another preferred embodiment, this method further includes the step of recovering the fine chemical from the culture. In a particularly preferred embodiment, the cell is from the genus *Corynebacterium* or *Brevibacterium*, or is selected from those strains set forth in Table 3.

Another aspect of the invention pertains to methods for modulating production of a molecule from a microorganism. Such methods include contacting the cell with an agent which modulates SES protein activity or SES nucleic acid expression such that a cell associated activity is altered relative to this same activity in the absence of the agent. In a preferred embodiment, the cell is modulated for one or more *C. glutamicum* processes involved in genetic stability, gene expression, protein folding, or protein secretion such that the yield, production, or efficiency of production of a desired fine chemical by this microorganism is improved. The agent which modulates SES protein activity can be an agent which stimulates SES protein activity or SES nucleic acid expression. Examples of agents which stimulate SES protein activity or SES nucleic acid expression include small molecules, active SES proteins, and nucleic acids encoding SES proteins that have been introduced into the cell. Examples of agents which inhibit SES activity or expression include small molecules and antisense SES nucleic acid molecules.

Another aspect of the invention pertains to methods for modulating yields of a desired compound from a cell, involving the introduction of a wild-type or mutant SES gene into a cell, either maintained on a separate plasmid or integrated into the genome of the host cell. If integrated into the genome, such integration can be random, or it can take place by homologous recombination such that the native gene is replaced by the introduced copy, causing the production of the desired compound from the cell to be

00320956 . 093300

modulated. In a preferred embodiment, said yields are increased. In another preferred embodiment, said chemical is a fine chemical. In a particularly preferred embodiment, said fine chemical is an amino acid. In especially preferred embodiments, said amino acid is L-lysine.

Detailed Description of the Invention

The present invention provides SES nucleic acid and protein molecules which are involved in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. The molecules of the invention may be utilized in the modulation of production of fine chemicals from microorganisms, such as *C. glutamicum*, either directly (*e.g.*, where overexpression or optimization of activity of a protein involved in secretion of a fine chemical (*e.g.*, an enzyme) has a direct impact on the yield, production, and/or efficiency of production of a fine chemical from the modified *C. glutamicum*), or an indirect impact which nonetheless results in an increase of yield, production, and/or efficiency of production of the desired compound (*e.g.*, where modulation of the activity or number of copies of a *C. glutamicum* DNA repair protein results in alterations in the ability of the microorganism to maintain the introduced mutation, which in turn may impact the production of one or more fine chemicals from such a strain). Aspects of the invention are further explicated below.

09622359.092900

I. Fine Chemicals

The term 'fine chemical' is art-recognized and includes molecules produced by an organism which have applications in various industries, such as, but not limited to, the pharmaceutical, agriculture, and cosmetics industries. Such compounds include organic acids, such as tartaric acid, itaconic acid, and diaminopimelic acid, both proteinogenic and non-proteinogenic amino acids, purine and pyrimidine bases, nucleosides, and nucleotides (as described *e.g.* in Kuninaka, A. (1996) Nucleotides and related compounds, p. 561-612, in Biotechnology vol. 6, Rehm et al., eds. VCH: Weinheim, and references contained therein), lipids, both saturated and unsaturated fatty acids (*e.g.*, arachidonic acid), diols (*e.g.*, propane diol, and butane diol), carbohydrates (*e.g.*, hyaluronic acid and trehalose), aromatic compounds (*e.g.*, aromatic amines, vanillin, and indigo), vitamins and cofactors (as described in Ullmann's Encyclopedia of Industrial Chemistry, vol. A27, "Vitamins", p. 443-613 (1996) VCH: Weinheim and references therein; and Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological

Associations in Malaysia, and the Society for Free Radical Research – Asia, held Sept. 1-3, 1994 at Penang, Malaysia, AOCS Press, (1995)), enzymes, polyketides (Cane *et al.*(1998) *Science* 282: 63-68), and all other chemicals described in Gutcho (1983) Chemicals by Fermentation, Noyes Data Corporation, ISBN: 0818805086 and references therein. The metabolism and uses of certain of these fine chemicals are further explicated below.

A. *Amino Acid Metabolism and Uses*

Amino acids comprise the basic structural units of all proteins, and as such are essential for normal cellular functioning in all organisms. The term "amino acid" is art-recognized. The proteinogenic amino acids, of which there are 20 species, serve as structural units for proteins, in which they are linked by peptide bonds, while the nonproteinogenic amino acids (hundreds of which are known) are not normally found in proteins (see Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97 VCH: Weinheim (1985)). Amino acids may be in the D- or L- optical configuration, though L-amino acids are generally the only type found in naturally-occurring proteins. Biosynthetic and degradative pathways of each of the 20 proteinogenic amino acids have been well characterized in both prokaryotic and eukaryotic cells (see, for example, Stryer, L. Biochemistry, 3rd edition, pages 578-590 (1988)). The 'essential' amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), so named because they are generally a nutritional requirement due to the complexity of their biosyntheses, are readily converted by simple biosynthetic pathways to the remaining 11 'nonessential' amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine). Higher animals do retain the ability to synthesize some of these amino acids, but the essential amino acids must be supplied from the diet in order for normal protein synthesis to occur.

Aside from their function in protein biosynthesis, these amino acids are interesting chemicals in their own right, and many have been found to have various applications in the food, feed, chemical, cosmetics, agriculture, and pharmaceutical industries. Lysine is an important amino acid in the nutrition not only of humans, but also of monogastric animals such as poultry and swine. Glutamate is most commonly used as a flavor additive (mono-sodium glutamate, MSG) and is widely used throughout the food industry, as are aspartate, phenylalanine, glycine, and cysteine. Glycine, L-methionine and tryptophan are all utilized in the pharmaceutical industry. Glutamine, valine, leucine, isoleucine, histidine, arginine, proline, serine and alanine are of use in both the pharmaceutical and cosmetics industries. Threonine, tryptophan, and D/ L-methionine are common feed additives. (Leuchtenberger, W. (1996) Amino aids –

technical production and use, p. 466-502 in Rehm *et al.*(eds.) Biotechnology vol. 6, chapter 14a, VCH: Weinheim). Additionally, these amino acids have been found to be useful as precursors for the synthesis of synthetic amino acids and proteins, such as N-acetylcysteine, S-carboxymethyl-L-cysteine, (S)-5-hydroxytryptophan, and others described in Ulmann's Encyclopedia of Industrial Chemistry, vol. A2, p. 57-97, VCH: Weinheim, 1985.

The biosynthesis of these natural amino acids in organisms capable of producing them, such as bacteria, has been well characterized (for review of bacterial amino acid biosynthesis and regulation thereof, see Umbarger, H.E.(1978) *Ann. Rev. Biochem.* 47: 533-606). Glutamate is synthesized by the reductive amination of α-ketoglutarate, an intermediate in the citric acid cycle. Glutamine, proline, and arginine are each subsequently produced from glutamate. The biosynthesis of serine is a three-step process beginning with 3-phosphoglycerate (an intermediate in glycolysis), and resulting in this amino acid after oxidation, transamination, and hydrolysis steps. Both cysteine and glycine are produced from serine; the former by the condensation of homocysteine with serine, and the latter by the transferal of the side-chain β-carbon atom to tetrahydrofolate, in a reaction catalyzed by serine transhydroxymethylase. Phenylalanine, and tyrosine are synthesized from the glycolytic and pentose phosphate pathway precursors erythrose 4-phosphate and phosphoenolpyruvate in a 9-step biosynthetic pathway that differ only at the final two steps after synthesis of prephenate. Tryptophan is also produced from these two initial molecules, but its synthesis is an 11-step pathway. Tyrosine may also be synthesized from phenylalanine, in a reaction catalyzed by phenylalanine hydroxylase. Alanine, valine, and leucine are all biosynthetic products of pyruvate, the final product of glycolysis. Aspartate is formed from oxaloacetate, an intermediate of the citric acid cycle. Asparagine, methionine, threonine, and lysine are each produced by the conversion of aspartate. Isoleucine is formed from threonine. A complex 9-step pathway results in the production of histidine from 5-phosphoribosyl-1-pyrophosphate, an activated sugar.

Amino acids in excess of the protein synthesis needs of the cell cannot be stored, and are instead degraded to provide intermediates for the major metabolic pathways of the cell (for review see Stryer, L. Biochemistry 3rd ed. Ch. 21 "Amino Acid Degradation and the Urea Cycle" p. 495-516 (1988)). Although the cell is able to convert unwanted amino acids into useful metabolic intermediates, amino acid production is costly in terms of energy, precursor molecules, and the enzymes necessary to synthesize them. Thus it is not surprising that amino acid biosynthesis is regulated by feedback inhibition, in which the presence of a particular amino acid serves to slow or entirely stop its own production (for overview of feedback mechanisms in amino acid biosynthetic pathways,

see Stryer, L. Biochemistry, 3rd ed. Ch. 24: "Biosynthesis of Amino Acids and Heme" p. 575-600 (1988)). Thus, the output of any particular amino acid is limited by the amount of that amino acid present in the cell.

B. Vitamin, Cofactor, and Nutraceutical Metabolism and Uses

Vitamins, cofactors, and nutraceuticals comprise another group of molecules which the higher animals have lost the ability to synthesize and so must ingest, although they are readily synthesized by other organisms such as bacteria. These molecules are either bioactive substances themselves, or are precursors of biologically active substances which may serve as electron carriers or intermediates in a variety of metabolic pathways. Aside from their nutritive value, these compounds also have significant industrial value as coloring agents, antioxidants, and catalysts or other processing aids. (For an overview of the structure, activity, and industrial applications of these compounds, see, for example, Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996.) The term "vitamin" is art-recognized, and includes nutrients which are required by an organism for normal functioning, but which that organism cannot synthesize by itself. The group of vitamins may encompass cofactors and nutraceutical compounds. The language "cofactor" includes nonproteinaceous compounds required for a normal enzymatic activity to occur. Such compounds may be organic or inorganic; the cofactor molecules of the invention are preferably organic. The term "nutraceutical" includes dietary supplements having health benefits in plants and animals, particularly humans. Examples of such molecules are vitamins, antioxidants, and also certain lipids (*e.g.*, polyunsaturated fatty acids).

The biosynthesis of these molecules in organisms capable of producing them, such as bacteria, has been largely characterized (Ullman's Encyclopedia of Industrial Chemistry, "Vitamins" vol. A27, p. 443-613, VCH: Weinheim, 1996; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley & Sons; Ong, A.S., Niki, E. & Packer, L. (1995) "Nutrition, Lipids, Health, and Disease" Proceedings of the UNESCO/Confederation of Scientific and Technological Associations in Malaysia, and the Society for Free Radical Research – Asia, held Sept. 1-3, 1994 at Penang, Malaysia, AOCS Press: Champaign, IL X, 374 S).

Thiamin (vitamin B_1) is produced by the chemical coupling of pyrimidine and thiazole moieties. Riboflavin (vitamin B_2) is synthesized from guanosine-5'-triphosphate (GTP) and ribose-5'-phosphate. Riboflavin, in turn, is utilized for the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The family of compounds collectively termed 'vitamin B_6' (*e.g.*, pyridoxine, pyridoxamine, pyridoxa-

5'-phosphate, and the commercially used pyridoxin hydrochloride) are all derivatives of the common structural unit, 5-hydroxy-6-methylpyridine. Pantothenate (pantothenic acid, (R)-(+)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine) can be produced either by chemical synthesis or by fermentation. The final steps in pantothenate biosynthesis consist of the ATP-driven condensation of β-alanine and pantoic acid. The enzymes responsible for the biosynthesis steps for the conversion to pantoic acid, to β-alanine and for the condensation to panthotenic acid are known. The metabolically active form of pantothenate is Coenzyme A, for which the biosynthesis proceeds in 5 enzymatic steps. Pantothenate, pyridoxal-5'-phosphate, cysteine and ATP are the precursors of Coenzyme A. These enzymes not only catalyze the formation of panthothante, but also the production of (R)-pantoic acid, (R)-pantolacton, (R)-panthenol (provitamin B_5), pantetheine (and its derivatives) and coenzyme A.

Biotin biosynthesis from the precursor molecule pimeloyl-CoA in microorganisms has been studied in detail and several of the genes involved have been identified. Many of the corresponding proteins have been found to also be involved in Fe-cluster synthesis and are members of the nifS class of proteins. Lipoic acid is derived from octanoic acid, and serves as a coenzyme in energy metabolism, where it becomes part of the pyruvate dehydrogenase complex and the α-ketoglutarate dehydrogenase complex. The folates are a group of substances which are all derivatives of folic acid, which is turn is derived from L-glutamic acid, p-amino-benzoic acid and 6-methylpterin. The biosynthesis of folic acid and its derivatives, starting from the metabolism intermediates guanosine-5'-triphosphate (GTP), L-glutamic acid and p-amino-benzoic acid has been studied in detail in certain microorganisms.

Corrinoids (such as the cobalamines and particularly vitamin B_{12}) and porphyrines belong to a group of chemicals characterized by a tetrapyrole ring system. The biosynthesis of vitamin B_{12} is sufficiently complex that it has not yet been completely characterized, but many of the enzymes and substrates involved are now known. Nicotinic acid (nicotinate), and nicotinamide are pyridine derivatives which are also termed 'niacin'. Niacin is the precursor of the important coenzymes NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) and their reduced forms.

The large-scale production of these compounds has largely relied on cell-free chemical syntheses, though some of these chemicals have also been produced by large-scale culture of microorganisms, such as riboflavin, Vitamin B_6, pantothenate, and biotin. Only Vitamin B_{12} is produced solely by fermentation, due to the complexity of its synthesis. *In vitro* methodologies require significant inputs of materials and time, often at great cost.

C. Purine, Pyrimidine, Nucleoside and Nucleotide Metabolism and Uses

Purine and pyrimidine metabolism genes and their corresponding proteins are important targets for the therapy of tumor diseases and viral infections. The language "purine" or "pyrimidine" includes the nitrogenous bases which are constituents of nucleic acids, co-enzymes, and nucleotides. The term "nucleotide" includes the basic structural units of nucleic acid molecules, which are comprised of a nitrogenous base, a pentose sugar (in the case of RNA, the sugar is ribose; in the case of DNA, the sugar is D-deoxyribose), and phosphoric acid. The language "nucleoside" includes molecules which serve as precursors to nucleotides, but which are lacking the phosphoric acid moiety that nucleotides possess. By inhibiting the biosynthesis of these molecules, or their mobilization to form nucleic acid molecules, it is possible to inhibit RNA and DNA synthesis; by inhibiting this activity in a fashion targeted to cancerous cells, the ability of tumor cells to divide and replicate may be inhibited. Additionally, there are nucleotides which do not form nucleic acid molecules, but rather serve as energy stores (*i.e.*, AMP) or as coenzymes (*i.e.*, FAD and NAD).

Several publications have described the use of these chemicals for these medical indications, by influencing purine and/or pyrimidine metabolism (*e.g.* Christopherson, R.I. and Lyons, S.D. (1990) "Potent inhibitors of *de novo* pyrimidine and purine biosynthesis as chemotherapeutic agents." *Med. Res. Reviews* 10: 505-548). Studies of enzymes involved in purine and pyrimidine metabolism have been focused on the development of new drugs which can be used, for example, as immunosuppressants or anti-proliferants (Smith, J.L., (1995) "Enzymes in nucleotide synthesis." *Curr. Opin. Struct. Biol.* 5: 752-757; (1995) *Biochem Soc. Transact.* 23: 877-902). However, purine and pyrimidine bases, nucleosides and nucleotides have other utilities: as intermediates in the biosynthesis of several fine chemicals (*e.g.*, thiamine, S-adenosyl-methionine, folates, or riboflavin), as energy carriers for the cell (*e.g.*, ATP or GTP), and for chemicals themselves, commonly used as flavor enhancers (*e.g.*, IMP or GMP) or for several medicinal applications (see, for example, Kuninaka, A. (1996) Nucleotides and Related Compounds in Biotechnology vol. 6, Rehm et al., eds. VCH: Weinheim, p. 561-612). Also, enzymes involved in purine, pyrimidine, nucleoside, or nucleotide metabolism are increasingly serving as targets against which chemicals for crop protection, including fungicides, herbicides and insecticides, are developed.

The metabolism of these compounds in bacteria has been characterized (for reviews see, for example, Zalkin, H. and Dixon, J.E. (1992) "*de novo* purine nucleotide biosynthesis", in: Progress in Nucleic Acid Research and Molecular Biology, vol. 42, Academic Press:, p. 259-287; and Michal, G. (1999) "Nucleotides and Nucleosides", Chapter 8 in: Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology,

09502345 - 050200

Wiley: New York). Purine metabolism has been the subject of intensive research, and is essential to the normal functioning of the cell. Impaired purine metabolism in higher animals can cause severe disease, such as gout. Purine nucleotides are synthesized from ribose-5-phosphate, in a series of steps through the intermediate compound inosine-5'-phosphate (IMP), resulting in the production of guanosine-5'-monophosphate (GMP) or adenosine-5'-monophosphate (AMP), from which the triphosphate forms utilized as nucleotides are readily formed. These compounds are also utilized as energy stores, so their degradation provides energy for many different biochemical processes in the cell. Pyrimidine biosynthesis proceeds by the formation of uridine-5'-monophosphate (UMP) from ribose-5-phosphate. UMP, in turn, is converted to cytidine-5'-triphosphate (CTP). The deoxy- forms of all of these nucleotides are produced in a one step reduction reaction from the diphosphate ribose form of the nucleotide to the diphosphate deoxyribose form of the nucleotide. Upon phosphorylation, these molecules are able to participate in DNA synthesis.

D. Trehalose Metabolism and Uses

Trehalose consists of two glucose molecules, bound in α, α-1,1 linkage. It is commonly used in the food industry as a sweetener, an additive for dried or frozen foods, and in beverages. However, it also has applications in the pharmaceutical, cosmetics and biotechnology industries (see, for example, Nishimoto et al., (1998) U.S. Patent No. 5,759,610; Singer, M.A. and Lindquist, S. (1998) *Trends Biotech.* 16: 460-467; Paiva, C.L.A. and Panek, A.D. (1996) *Biotech. Ann. Rev.* 2: 293-314; and Shiosaka, M. (1997) J. Japan 172: 97-102). Trehalose is produced by enzymes from many microorganisms and is naturally released into the surrounding medium, from which it can be collected using methods known in the art.

II. Genetic Stability; Protein Synthesis and Protein Secretion in *C. glutamicum*

The production of a desired compound from a cell such as *C. glutamicum* is the culmination of a large number of separate yet interrelated processes, each of which is critical to the overall production and release of the compound from the cell. In engineering a cell to overproduce one or more fine chemicals, consideration must be given to each of these processes to ensure that the biochemical machinery of the cell will be compatible with such genetic manipulation. Cellular mechanisms of particular importance include the stability of the altered gene(s) upon introduction into the cell, the ability of the mutated gene to be properly transcribed and translated (including issues of codon usage), and the ability of the mutant protein product to be appropriately folded and/or secreted.

Docket No. BGI-127CP

A. Bacterial Repair and Recombination Systems

Cells are constantly exposed to nucleic acid-damaging agents, such as UV irradiation, oxygen radicals, and alkylation. Further, even the action of DNA polymerases is not error-free. Cells must maintain a balance between genetic stability (which ensures that genes necessary for vital cellular functions are not damaged during normal growth and metabolism) and genetic variability (which permits cells to adapt to a changing environment). Therefore, there exist separate, but interrelated pathways of DNA repair and DNA recombination in most cells. The former serves to stringently correct errors in DNA molecules by either directly reversing the damage or excising the damaged region and replacing it with the correct sequence. The latter recombination system also repairs nucleic acid molecules, but only those lesions that result in damage to both strands of DNA such that neither strand is able to serve as a template to correct the other. Recombination repair and the SOS response may readily lead to inversions, deletions, or other genetic rearrangements within or around the region of the damage, which in turn promotes a certain degree of genomic instability which may contribute to the ability of the cell to adapt to changing environments or stresses.

High-fidelity repair mechanisms include direct reversal of DNA damage and excision of damage and resynthesis using the information encoded on the opposite DNA strand. Direct reversal of damage requires an enzyme having an activity opposite of that which originally damaged the DNA. For example, inappropriate methylation of DNA may be corrected by the action of DNA repair methyltransferases, and nucleotide dimers created by UV irradiation may be fixed by the activity of deoxyribodipyrimidine photo-lyase, which, in the presence of light, cleaves the dimer back to its constituent nucleotides (see Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, Wiley: New York, and references therein).

Precise repair of more extensive damage requires specialized repair mechanisms. These include the mismatch repair and excision repair systems. Damage to a single base may be corrected by a series of cleavage reactions, where first the sugar-base bond is cut, followed by cleavage of the DNA backbone at the site of damage and removal of the damaged base itself. Finally, DNA polymerase and DNA ligase act to fill in and seal the gap using the second DNA strand as a template. More significant DNA damage which results in altered conformation of the double helix is corrected by the ABC system, in which helicase II, DNA polymerase I, UvrA, UvrB, and UvrC proteins combine to nick the double helix at the site of damage, to unwind the damaged region in an ATP-dependent fashion, to excise the damaged region, and to fill in the missing region using the other strand as a template. Lastly, DNA ligase seals the nick. Specific repair systems also exist for G·T mismatches (involving the Vsr protein) and for small

Docket No. BGI-127CP

deletion/insertion errors resulting in mispairing of the two strands (involving the methylation-directed pathway).

There also exist low-fidelity repair systems which are generally used to correct very extensive DNA damage in bacteria. Double-strand repair and recombination occurs in the presence of a lesion which affects both strands of DNA. In this situation, it is impossible to repair the damage utilizing the other strand as the template. Thus, this repair system involves a double-crossover event between the area of the lesion and another copy of the region on a homologous DNA molecule. This is possible because bacteria divide so rapidly that a second copy of genomic DNA is usually available before actual cell division occurs. This crossover event may readily lead to inversions, duplications, deletions, insertions and other genetic rearrangements, and thus increases the overall genetic instability of the organism.

The SOS response is activated when sufficient damage is present in the DNA that DNA polymerase III stalls and cannot continue replication. Under these circumstances, single-stranded DNA is present. The RecA protein is activated by binding to single-stranded DNA, and this activated form results in the activation of the LexA repressor, thereby lifting the transcriptional block of more than 20 genes, including UvrA, UvrB, UvrC, helicase II, DNA pol III, UmuC, and UmuD. The combined activities of these enzymes results in sufficient filling of the gap region that DNA pol III is able to resume replication. However, these gaps have been filled in with bases which should not be present; thus, this type of repair results in error-prone repair, contributing to overall genetic instability in the cell.

B. Transposons

The aforementioned systems, whether high or low fidelity, exist to repair DNA damage. In certain circumstances, this repair may accidentally incorporate additional genetic rearrangements. Many bacterial cells also have mechanisms specifically designed to cause such genetic rearrangements. Particularly well-known examples of such mechanisms are the transposons.

Transposons are genetic elements which are able to move from one site to another either within a chromosome or between a piece of extrachromosomal DNA (*e.g.*, a plasmid) and a chromosome. Transposition may occur in multiple ways; for example, the transposable element may be cut out from the donor site and integrated into the target site (nonreplicative transposition), or the transposable element may alternately be duplicated from the donor site to the target site, yielding two copies of the element (replicative transposition). There is generally no sequence relationship between the donor and target sites.

There are a variety of results possible from such a transposition event. The integration of a transposable element into a gene disrupts the gene, usually abrogating its function entirely. An integration event that occurs in the DNA surrounding a gene may not perturb the coding sequence itself, but can have a profound effect on the regulation of the gene and thus, on its expression. Recombination events between two copies of a transposable element found in different portions of the genome may result in deletions, duplications, inversions, transpositions, or amplifications of segments of the genome. It is also possible for different replicons to fuse.

The simplest transposon-like genetic elements are termed insertion (IS) elements. IS elements contain a nucleotide region of varying length (though usually less than 1500 bases) lacking any coding regions, surrounded by inverted repeats at either end. Thus, since the IS element does not encode any proteins whose activity may be detected, the presence of an IS element is generally only observed due to a loss of function of one or more genes in which the IS element is inserted.

Transposons are mobile genetic elements which, unlike IS elements, contain nucleic acid sequences bounded by repeats which may encode one or more proteins. It is not unusual for these repeat regions to consist of IS elements. The proteins encoded by the transposon are typically transposases (proteins which catalyze the movement of the transposon from one site to another) and antibiotic resistance genes. The mechanisms and regulation of transposable elements are well known in the art and are have been described at least in, for example, Lengeler *et al.*(1999) Biology of Prokaryotes, Thieme Verlag: Stuttgart, p. 375-361; Neidhardt *et al.*(1996) *Escherichia coli* and *Salmonella*, ASM Press: Washington, D.C.; Sonenshein, A.L. et al., eds. (1993), *Bacillus subtilis*, ASM Press: Washington, D.C.; Voet, D. and Voet, J.G. (1992) Biochemie, VCH: Weinheim, p. 985-990; Brock, T.D., and Madigan, M.T. (1991) Biology of Microorganisms, 6th ed., Prentice Hall: New York, p. 267-269; and Kleckner, N. (1990) "Regulation of transposition in bacteria", *Annu. Rev. Biochem.* 61: 297-327.

C. Transcription

Gene expression in bacteria is regulated mainly at the level of transcription. The transcriptional apparatus consists of a number of proteins that can be divided into two groups: RNA polymerase (the processive DNA-transcribing enzyme) and sigma factors (which regulate gene transcription by directing RNA polymerase to specific promoter-DNA sequences which these factors recognize). The combination of RNA polymerase and sigma factors creates the RNA polymerase holoenzyme, an activated complex. Gram positive bacteria such as Corynebacteria contain only one type of RNA-polymerase, but a variety of different sigma factors specific for different promoters,

growth phases, environmental conditions, substrates, oxygen levels, transport processes, and the like, which permits adaptability of the organism to different environmental and metabolic conditions.

Promoters are specific DNA sequences that serve as docking sites for the RNA polymerase holoenzyme. Many promoter elements possess conserved sequence elements that may be recognized through homology searches; alternately, promoter regions for a particular gene may be identified using standard techniques such as primer extension. Many promoter regions from gram-positive bacteria are known (see, *e.g.*, Sonenshein, A.L., Hoch, J.A., and Losick, R., eds. (1993) *Bacillus subtilis*, ASM Press: Washington, D.C.).

Promoter transcriptional control is influenced by several mechanisms of repression or activation. Specific regulatory proteins which bind promoters have the ability to block (repressors) or to assist (activators) the binding of the RNA holoenzyme, and thus to regulate transcription. The binding of these repressor and activator molecules in turn is regulated by their interactions with other molecules, such as proteins or other metabolic compounds. Transcription may alternately be regulated by factors influencing processes such as elongation or termination (see, *e.g.*, Sonenshein, A.L., Hoch, J.A., and Losick, R., eds. (1993) *Bacillus subtilis*, ASM Press: Washington, D.C.). The ability to regulate transcription of genes in response to a variety of environmental or metabolic cues affords cells the ability to tightly control when a gene may be expressed and or how much of a gene product may be present in the cell at one time. This in turn prevents unnecessary expenditure of energy or unnecessary utilization of possibly scarce intermediate compounds or cofactors.

D. Translation and tRNA-Aminoacyl Synthetases

Translation is the process by which a polypeptide is synthesized from amino acids according to the information contained within an mRNA molecule. The main components of this process are ribosomes and specific initiation or elongation factors, such as IF1-3, EF-G, and EFTu (see, *e.g.*, Sonenshein, A.L., Hoch, J..A., Losick, R., eds. (1993) *Bacillus subtilis*, ASM Press: Washington, D.C.).

Each codon of the mRNA molecule encodes a particular amino acid. The conversion from mRNA to amino acid is effected by transfer RNA (tRNA) molecules. These molecules consist of a single strand of RNA (between 60 and 100 bases), which exists in an L-shaped three dimensional structure having protruding areas, or 'arms'. One such arm forms base pairs with a particular codon sequence on the mRNA molecule. A second arm interacts specifically with a particular amino acid (the one encoded by the codon). Other arms of the tRNA include the variable arm, the TψC arm

(which bears thimidylate and pseudouridylate modifications), and the D arm (which bears a dihydrouridine modification). The function of these latter structures remains unknown, but their conservation between tRNA molecules suggests a role in protein synthesis.

In order for the nucleic acid-based tRNA molecule to associate with the correct amino acid, a family of enzymes, termed the aminoacyl-tRNA synthetases, must act. There exist many different of these enzymes, each of which is specific for a particular tRNA and a particular amino acid. These enzymes link the 3' hydroxyl of the terminal tRNA adenosine ribose moiety to the amino acid in a two step reaction. First, the enzyme is activated by reaction with ATP and the amino acid to result in an aminoacyl-tRNA synthetase-aminoacyl adenylate complex. Second, the aminoacyl group is transferred from the enzyme to the target tRNA where it remains in the high-energy state. Binding of the tRNA molecule to its cognate codon on the mRNA molecule then brings the high-energy amino acid attached to the tRNA into contact with the ribosome. Within the ribosome, the amino-acid charged tRNA (aminoacyl-tRNA) occupies one binding site (the A site) adjacent to a second site (the P site) containing a tRNA molecule whose amino acid arm is attached to the nascent polypeptide chain (peptidyl-tRNA). The activated amino acid on the aminoacyl-tRNA is sufficiently reactive that a peptide bond spontaneously forms between this amino acid and the next amino acid on the nascent polypeptide chain. Hydrolysis of GTP provides the energy for the transfer of the now-polypeptide chain-loaded tRNA from the A site to the P site of the ribosome, and the process repeats until a stop codon is reached.

There are a number of different steps at which translation may be regulated. These include the binding of the ribosome to mRNA, the presence of mRNA secondary structure, codon usage, or the abundance of particular tRNAs. Also, special regulation mechanisms such as attenuation may act at the level of translation. For an in-depth review of many of these mechanisms, see, *e.g.*, Vellanoweth, R.L. (1993) "Translation and its Regulation" in: *Bacillus subtilis* and other Gram Positive Bacteria, Sonenshein, A.L. et al., eds., ASM Press: Washington D.C., p. 699-711, and references cited therein.

E. Protein Folding and Secretion

Synthesis of proteins by the ribosome results in polypeptide chains, which must take on a three-dimensional form before the protein can function normally. This three-dimensional structure is achieved by a process of folding. Polypeptide chains are flexible, and (in principle) move readily and freely in solution until they attain a conformation which results in a stable three-dimensional structure. However, it is sometimes difficult for proteins to fold correctly, either due to environmental conditions

(*e.g.*, high temperature, where the extra kinetic energy present in the system makes it more difficult for the polypeptide to settle in the energy well of a stable structure) or due to the nature of the protein itself (*e.g.*, the hydrophobic regions in nearby polypeptides have a tendency to aggregate and thereby sequester themselves from aqueous solution).

Proteinaceous factors have been identified that are able to catalyze, chaperone, or otherwise assist in the folding of proteins being synthesized either co- or posttranslationally. Members of these protein folding molecules are the prolyl-peptidyl isomerases (*e.g.*, trigger factor, cyclophilin, and FKBP homologs), and proteins of the heat shock protein group (*e.g.*, DnaK, DnaJ, GroEL, small heat shock proteins, HtpG and members of the Clp family (*e.g.*, ClpA, ClpB, ClpW, ClpP, and ClpX)). Many of these proteins are essential for the viability of cells: in addition to their functions in protein folding, translocation, and processing, they frequently serve as key targets for the overall regulation of protein synthesis (see, *e.g.*, Bukau, B., (1993) *Molecular Microbiology* 9(4): 671-680; Bukau, B., and Horwich, A.L. (1998) *Cell* 92(3):351-366; Hesterkamp, T., Bukau, C. (1996) *FEBS Lett.* 389(1):32-34; Yaron, A., Naider, F. (1993) *Critical Reviews in Biochemistry and Molecular Biology* 28(1):31-81; Scheibel, R., Buchner, J. (1998) *Biochemical Pharmacology* 56(6):675-682; Ellis, R.J., Hartl, F.U. (1996) *FASEB Journal* 10(1): 20-26; Wawrzynow, A. *et al.*(1996) *Molecular Microbiology* 21(5): 895-899; Ewalt, K.L., *et al.*(1997) *Cell* 90(3): 491-500).

Chaperones identified thus far function in one of two ways: they either bind and stabilize polypeptides, or they provide an environment in which folding may occur without interference. The former group, including, *e.g.*, DnaK, DnaJ, and the heat shock proteins, bind directly to the nascent or misfolded polypeptide, frequently with concomitant ATP hydrolysis. The association of the chaperone prevents the polypeptide from aggregating with other polypeptides, and can force such aggregates to dissipate if they have already formed. After interaction with a second chaperone, GrpE (which permits an ADP-ATP exchange to occur), the polypeptide is released in a molten globule state and is permitted to fold. If misfolding occurs, the chaperones again associate with the misfolded protein, forcing it to return to an unfolded state. This cycle may be repeated until the protein is correctly folded. Unlike the first type of chaperones, which simply bind to the polypeptide, the second group (*e.g.* GroEL/ES) not only bind to the polypeptide, but also completely surround it such that it is protected from the surrounding environment. The GroEL/ES complex is composed of 2 stacked 14-member rings having a hydrophobic interior surface, and a 7-membered ring 'cap'. The polypeptide is drawn into the channel in the center of this complex in an ATP-dependent reaction where it is able to fold without interference from other polypeptides. Incorrectly folded proteins are not released from the complex.

An important step in protein folding is the creation of disulfide bonds. These bonds, either within a subunit or between subunits of a protein, are critical for protein stability. Disulfide bonds form readily in aqueous solution, and incorrect disulfide bond formation is difficult to reverse without the aid of a reducing environment. To assist in this process of correct disulfide bond formation, thiol-containing molecules, such as glutathione or thioredoxin, and their respective oxidation/reduction systems are found in the cytosol of most cells (Loferer, H., Hennecke, H. (1994) *Trends in Biochemical Sciences* 19(4): 169-171).

There are times, however, when folding of nascent polypeptide chains is not desirable, such as when these polypeptides are to be secreted. The folding process generally results in the hydrophobic regions of the protein being in the center of the protein, away from aqueous solution, and the hydrophilic regions being presented at the outer surfaces of the protein. This conformational arrangement, while creating greater stability for the protein, makes it difficult for the protein to be translocated across membranes, since the hydrophobic core of the membrane is inherently incompatible with the hydrophilic exterior of the protein. Thus, proteins synthesized by the cell which must be secreted to the exterior of the cell (*e.g.*, cell surface enzymes and membrane receptors) or which must be inserted into the membrane itself (*e.g.*, transporter proteins and channel proteins) are generally secreted or inserted prior to folding. The same chaperones which prevent aggregation of nascent polypeptide chains also prevent folding of polypeptides until they are disengaged. Thus, these proteins may 'escort' nascent polypeptide chains to an appropriate cellular location where they either are removed, thereby permitting folding, or they transfer the polypeptide to a transport system which will either secrete the polypeptide or aid its insertion into a membrane.

A specialized protein machinery has evolved that specifically detects, binds, transports, and processes proteins bearing specific prosequences (these sequences are later removed from the protein by cleavage). This machinery consists of a number of proteins which are collectively termed the sec (type II secretion) system (for review, see Gilbert, M. *et al.*(1995) *Critical Reviews in Biotechnology* 15(1): 13-39 and references therein; Freudl, R. (1992) *Journal of Biotechnology* 23(3): 231-240 and references therein; Neidhardt, F.C. *et al.*(1996) *E. coli* and *Salmonella* ASM Press: Washington, D.C., p. 967-978; Binet, R. *et al.*(1997) *Gene* 192(1): 7-11; and Rapoport, T.A. (1986) *Critical Reviews in Biochemistry* 20(1): 73-137, and references therein). The sec system is composed of chaperones (*e.g.*, SecA and SecB), integral membrane proteins, also called translocases (*e.g.*, SecY, SecE, and SecG), and signal peptidases (*e.g.*, LepB). The nascent polypeptide having a prosequence directing secretion is bound by SecB, which delivers it to SecA at the inner surface of the cell membrane. Sec A binds to the

prosequence and, upon ATP hydrolysis, inserts into the membrane and forces a portion of the polypeptide through the membrane as well. The remainder of the polypeptide is guided through the membrane by a complex of translocases, such as SecY, SecE, and SecG. Finally, the signal peptidase cleaves off the prosequence and the polypeptide is free on the extracellular side of the membrane, where it spontaneously folds.

Sec-independent secretion mechanisms are also known. For example, the signal recognition particle-dependent pathway involves the binding of a signal recognition particle (SRP) protein to the nascent polypeptide as it is being synthesized, forcing the ribosome to stall. A receptor for SRP at the inner surface of the membrane then binds the ribosome-polypeptide-SRP complex. Hydrolysis of GTP provides the energy necessary to transfer the complex to the sec translocase complex, where the nascent polypeptide is guided across the membrane as it is synthesized by the ribosome. Other secretion mechanisms specific to only a few proteins are also known to exist.

09602839.062300

III. Elements and Methods of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as SES nucleic acid and protein molecules, which participate in *C. glutamicum* DNA repair or recombination, in the transposition or other rearrangement of *C. glutamicum* DNA, in *C. glutamicum* gene expression (*e.g.*, the processes of transcription or translation), or in protein folding or protein secretion from this microorganism. In one embodiment, the SES molecules participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. In a preferred embodiment, the activity of the SES molecules of the present invention with regard to DNA repair or recombination, transposition of DNA, gene expression, protein folding or protein secretion has an impact on the production of a desired fine chemical by this organism. In a particularly preferred embodiment, the SES molecules of the invention are modulated in activity, such that the *C. glutamicum* cellular processes in which the SES molecules participate (*e.g.*, DNA repair or recombination, transposition of DNA, gene expression, protein folding, or protein secretion) are also altered in activity, resulting either directly or indirectly in a modulation of the yield, production, and/or efficiency of production of a desired fine chemical by *C. glutamicum*.

The language, "SES protein" or "SES polypeptide" includes proteins which participate in a number of cellular processes related to *C. glutamicum* genetic stability, gene expression, protein folding, or protein secretion. For example, an SES protein may be involved in *C. glutamicum* DNA repair or recombination mechanisms, in

rearrangements of *C. glutamicum* genetic material (such as those mediated by transposons), in transcription or translation of genes in this microorganism, in the mediation of *C. glutamicum* protein folding (such as the activity of chaperones) or in secretion of proteins from *C. glutamicum* cells (*e.g.*, the sec system). Examples of SES proteins include those encoded by the SES genes set forth in Table 1 and Appendix A. The terms "SES gene" or "SES nucleic acid sequence" include nucleic acid sequences encoding an SES protein, which consist of a coding region and also corresponding untranslated 5' and 3' sequence regions. Examples of SES genes include those set forth in Table 1. The terms "production" or "productivity" are art-recognized and include the concentration of the fermentation product (for example, the desired fine chemical) formed within a given time and a given fermentation volume (*e.g.*, kg product per hour per liter). The term "efficiency of production" includes the time required for a particular level of production to be achieved (for example, how long it takes for the cell to attain a particular rate of output of a fine chemical). The term "yield" or "product/carbon yield" is art-recognized and includes the efficiency of the conversion of the carbon source into the product (*i.e.*, fine chemical). This is generally written as, for example, kg product per kg carbon source. By increasing the yield or production of the compound, the quantity of recovered molecules, or of useful recovered molecules of that compound in a given amount of culture over a given amount of time is increased. The terms "biosynthesis" or a "biosynthetic pathway" are art-recognized and include the synthesis of a compound, preferably an organic compound, by a cell from intermediate compounds in what may be a multistep and highly regulated process. The terms "degradation" or a "degradation pathway" are art-recognized and include the breakdown of a compound, preferably an organic compound, by a cell to degradation products (generally speaking, smaller or less complex molecules) in what may be a multistep and highly regulated process. The language "metabolism" is art-recognized and includes the totality of the biochemical reactions that take place in an organism. The metabolism of a particular compound, then, (*e.g.*, the metabolism of an amino acid such as glycine) comprises the overall biosynthetic, modification, and degradation pathways in the cell related to this compound. The term "DNA repair" is art-recognized and includes cellular mechanisms whereby errors in DNA (due either to damage, such as, but not limited to, ultraviolet radiation, methylases, low-fidelity replication, or mutagens) are excised and corrected. The term "recombination" or "DNA recombination" is art-recognized and includes cellular mechanisms whereby extensive DNA damage affecting both strands of a DNA molecule is corrected by homologous recombination with another, undamaged copy of the DNA molecule within the same cell. Such repairs are generally low-fidelity, and may result in genetic rearrangements.

09622356.062300

The term "transposon" is art-recognized and includes a DNA element which is able to insert randomly throughout the genome of an organism, and which may result in the disruption of genes or their regulatory regions, or in duplications, inversions, deletions, and other genetic rearrangements. The term "protein folding" is art-recognized and includes the movement of a polypeptide chain through multiple three-dimensional configurations until the stable, active, three-dimensional configuration is attained. The formation of disulfide bonds and the sequestration of hydrophobic regions from the surrounding aqueous solution provide some of the driving forces for this folding process, and correct folding may be enhanced by the activity of chaperones. The terms "secretion" or "protein secretion" is art-recognized and includes the movement of proteins from the interior of the cell to the exterior of the cell, in a mechanism whereby a system of secretion proteins permits their transit across the cellular membrane to the exterior of the cell.

In another embodiment, the SES molecules of the invention are capable of modulating the production of a desired molecule, such as a fine chemical, in a microorganism such as *C. glutamicum*. There are a number of mechanisms by which the alteration of an SES protein of the invention may directly affect the yield, production, and/or efficiency of production of a fine chemical from a *C. glutamicum* strain incorporating such an altered protein. For example, modulation of proteins involved directly in transcription or translation (*e.g.*, polymerases or ribosomes) such that they are increased in number or in activity should increase global cellular transcription or translation (or rates of these processes). This increased cellular gene expression should include those proteins involved in fine chemical biosynthesis, so an increase in yield, production, or efficiency of production of one or more desired compounds may occur. Modifications to the transcriptional/translational protein machinery of *C. glutamicum* such that the regulation of these proteins is altered may also permit increased expression of genes involved in the production of fine chemicals. Modulation of the activity or number of proteins involved in polypeptide folding may permit an increase in the overall production of correctly folded molecules in the cell, thereby increasing the possibility that desired proteins (*e.g.*, fine chemical biosynthetic proteins) are able to function properly. Further, by mutating proteins involved in secretion from *C. glutamicum* such that they are increased in number or activity, it may be possible to increase the secretion of a fine chemical (*e.g.*, an enzyme) from cells in fermentor culture, where it may be readily recovered.

Genetic modification of the SES molecules of the invention may also result in indirect modulation of production of one or more fine chemicals. For example, by increasing the number or activity of a DNA repair or recombination protein of the

invention, one may increase the ability of the cell to detect and repair DNA damage. This should effectively increase the ability of the cell to maintain a mutated gene within its genome, thereby increasing the likelihood that a transgene engineered into ~~*into*~~ *C. glutamicum* (*e.g.*, encoding a protein which will increase biosynthesis of a fine chemical) will not be lost during culture of the microorganism. Conversely, by decreasing the number or activity of one or more DNA repair or recombination proteins, it may be possible to increase the genetic instability of the organism. Such manipulations should improve the ability of the organism to be modified by mutagenesis without the introduced mutation being corrected. The same holds true for proteins involved in transposition or rearrangement of genetic elements in *C. glutamicum* (*e.g.*, transposons). By mutagenizing these proteins such that they are either increased or decreased in number or activity, it is possible to simultaneously increase or decrease the genetic stability of the microorganism. This has a profound impact on the ability of any other mutation to be introduced into *C. glutamicum*, and on the ability of introduced mutations to be retained. Transposons also offer a convenient mechanism by which mutagenesis of *C. glutamicum* may be performed; duplication of desired genes (*e.g.*, fine chemical biosynthetic genes) is readily accomplished by transposon mutagenesis, as is disruption of undesired genes (*e.g.*, genes encoding proteins involved in degradation of desired fine chemicals).

By modulating one or more proteins (*e.g.* sigma factors) involved in the regulation of transcription or translation in response to particular environmental conditions, it may be possible to prevent the cell from slowing or stopping protein synthesis under unfavorable environmental conditions, such as those found in large-scale fermentor culture. This should lead to increased gene expression, which in turn may permit increased biosynthesis of desired fine chemicals under such conditions. Many such secreted proteins have functions critical for cell viability (*e.g.*, cell surface proteases or receptors). An alteration of a secretory pathway such that these proteins are more readily transported to their extracellular location may improve the overall viability of the cell, and thus result in greater numbers of *C. glutamicum* cells capable of producing fine chemicals during large-scale culture. Further, since certain bacterial protein secretion pathways (*e.g.*, the sec system) are known to participate in the insertion of integral membrane proteins (such as receptors, channels, pores, or transporters) into the membrane, the modulation of activity of proteins involved in protein secretion from *C. glutamicum* may affect the ability of the cell to excrete waste products or to import necessary metabolites. If the activity of these secretory proteins is increased, then the ability of the cell to produce fine chemicals may be similarly increased (due to an increase in the presence of transporters/channels in the membrane which may import

nutrients or excrete waste products). If the activity of these proteins is decreased, then there may be insufficient nutrients available to support overproduction of desired compounds, or waste products may interfere with fine chemical biosynthesis.

The isolated nucleic acid sequences of the invention are contained within the genome of a *Corynebacterium glutamicum* strain available through the American Type Culture Collection, given designation ATCC 13032. The nucleotide sequence of the isolated *C. glutamicum* SES DNAs and the predicted amino acid sequences of the *C. glutamicum* SES proteins are shown in Appendices A and B, respectively. Computational analyses were performed which classified and/or identified these nucleotide sequences as sequences which encode proteins involved in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum.*

The present invention also pertains to proteins which have an amino acid sequence which is substantially homologous to an amino acid sequence of Appendix B. As used herein, a protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence is least about 50% homologous to the selected amino acid sequence, *e.g.*, the entire selected amino acid sequence. A protein which has an amino acid sequence which is substantially homologous to a selected amino acid sequence can also be least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80%, 80-90%, or 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to the selected amino acid sequence.

The SES protein or a biologically active portion or fragment thereof of the invention can participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or have one or more of the activities set forth in Table 1.

Various aspects of the invention are described in further detail in the following subsections:

A. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode SES polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes or primers for the identification or amplification of SES-encoding nucleic acid (*e.g.*, SES DNA). As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic

DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. This term also encompasses untranslated sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 100 nucleotides of sequence upstream from the 5' end of the coding region and at least about 20 nucleotides of sequence downstream from the 3'end of the coding region of the gene. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated SES nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived (e.g, a *C. glutamicum* cell). Moreover, an "isolated" nucleic acid molecule, such as a DNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having a nucleotide sequence of Appendix A, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, a *C. glutamicum* SES DNA can be isolated from a *C. glutamicum* library using all or portion of one of the sequences of Appendix A as a hybridization probe and standard hybridization techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Moreover, a nucleic acid molecule encompassing all or a portion of one of the sequences of Appendix A can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence (*e.g.*, a nucleic acid molecule encompassing all or a portion of one of the sequences of Appendix A can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this same sequence of Appendix A). For example, mRNA can be isolated from normal endothelial cells (*e.g.*, by the guanidinium-thiocyanate extraction procedure of Chirgwin *et al.*(1979) *Biochemistry* 18: 5294-5299) and DNA can be prepared using reverse transcriptase (*e.g.*, Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be

designed based upon one of the nucleotide sequences shown in Appendix A. A nucleic acid of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to an SES nucleotide sequence can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises one of the nucleotide sequences shown in Appendix A. The sequences of Appendix A correspond to the *Corynebacterium glutamicum* SES DNAs of the invention. This DNA comprises sequences encoding SES proteins (*i.e.*, the "coding region", indicated in each sequence in Appendix A), as well as 5' untranslated sequences and 3' untranslated sequences, also indicated in Appendix A. Alternatively, the nucleic acid molecule can comprise only the coding region of any of the sequences in Appendix A.

For the purposes of this application, it will be understood that each of the sequences set forth in Appendix A has an identifying RXA, RXN, or RXS number having the designation "RXA", "RXN", or "RXS" followed by 5 digits (*i.e.*, RXA01278, RXN01559, or RXS00061). Each of these sequences comprises up to three parts: a 5' upstream region, a coding region, and a downstream region. Each of these three regions is identified by the same RXA, RXN, or RXS designation to eliminate confusion. The recitation "one of the sequences in Appendix A", then, refers to any of the sequences in Appendix A, which may be distinguished by their differing RXA, RXN, or RXS designations. The coding region of each of these sequences is translated into a corresponding amino acid sequence, which is set forth in Appendix B. The sequences of Appendix B are identified by the same RXA, RXN, or RXS designations as Appendix A, such that they can be readily correlated. For example, the amino acid sequences in Appendix B designated RXA01278, RXN01559, and RXS00061 are translations of the coding regions of the nucleotide sequence of nucleic acid molecules RXA01278, RXN01559, and RXS00061 respectively, in Appendix A. Each of the RXA, RXN, and RXS nucleotide and amino acid sequences of the invention has also been assigned a SEQ ID NO, as indicated in Table 1. For example, as set forth in Table 1, the nucleotide sequence of RXN01559 is SEQ ID NO:5, and the amino acid sequence of RXN01559 is SEQ ID NO:6.

Several of the genes of the invention are "F-designated genes". An F-designated gene includes those genes set forth in Table 1 which have an 'F' in front of the RXA, RXN or RXS designation. For example, SEQ ID NO:7, designated, as indicated on

Table 1, as "F RXA00935", is an F-designated gene, as are SEQ ID NOs: 9, 29, and 37 (designated on Table 1 as "F RXA01559", "F RXA00484", and "F RXA01670", respectively).

In one embodiment, the nucleic acid molecules of the present invention are not intended to include those compiled in Table 2. In the case of the dapD gene, a sequence for this gene was published in Wehrmann, A., *et al.*(1998) *J. Bacteriol.* 180(12): 3159-3165. However, the sequence obtained by the inventors of the present application is significantly longer than the published version. It is believed that the published version relied on an incorrect start codon, and thus represents only a fragment of the actual coding region.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of one of the nucleotide sequences shown in Appendix A, or a portion thereof. A nucleic acid molecule which is complementary to one of the nucleotide sequences shown in Appendix A is one which is sufficiently complementary to one of the nucleotide sequences shown in Appendix A such that it can hybridize to one of the nucleotide sequences shown in Appendix A, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleotide sequence which is at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%, preferably at least about 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%,and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to a nucleotide sequence shown in Appendix A, or a portion thereof. Ranges and identity values intermediate to the above-recited ranges, (*e.g.*, 70-90% identical or 80-95% identical) are also intended to be encompassed by the present invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. In an additional preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleotide sequence which hybridizes, *e.g.*, hybridizes under stringent conditions, to one of the nucleotide sequences shown in Appendix A, or a portion thereof.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the coding region of one of the sequences in Appendix A, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of an SES protein. The nucleotide sequences determined from the cloning of the SES genes from *C. glutamicum* allows for the generation of probes and primers

designed for use in identifying and/or cloning SES homologues in other cell types and organisms, as well as SES homologues from other *Corynebacteria* or related species. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense strand of one of the sequences set forth in Appendix A, an anti-sense sequence of one of the sequences set forth in Appendix A, or naturally occurring mutants thereof. Primers based on a nucleotide sequence of Appendix A can be used in PCR reactions to clone SES homologues. Probes based on the SES nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells which misexpress an SES protein, such as by measuring a level of an SES-encoding nucleic acid in a sample of cells, *e.g.*, detecting SES mRNA levels or determining whether a genomic SES gene has been mutated or deleted.

In one embodiment, the nucleic acid molecule of the invention encodes a protein or portion thereof which includes an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. As used herein, the language "sufficiently homologous" refers to proteins or portions thereof which have amino acid sequences which include a minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain as an amino acid residue in one of the sequences of Appendix B) amino acid residues to an amino acid sequence of Appendix B such that the protein or portion thereof is able to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. Proteins involved in *C. glutamicum* genetic stability, gene expression, protein folding or protein secretion, as described herein, may play a role in the production and secretion of one or more fine chemicals. Examples of such activities are also described herein. Thus, "the function of an SES protein" contributes either directly or indirectly to the yield, production, and/or efficiency of production of one or more fine chemicals. Examples of SES protein activities are set forth in Table 1.

In another embodiment, the protein is at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-80%, 80-90%, 90-95%, and most preferably at least about 96%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of Appendix B.

Portions of proteins encoded by the SES nucleic acid molecules of the invention are preferably biologically active portions of one of the SES proteins. As used herein, the term "biologically active portion of an SES protein" is intended to include a portion, *e.g.*, a domain/motif, of an SES protein that participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or has an activity as set forth in Table 1. To determine whether an SES protein or a biologically active portion thereof can participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, an assay of enzymatic activity may be performed. Such assay methods are well known to those of ordinary skill in the art, as detailed in Example 8 of the Exemplification.

Additional nucleic acid fragments encoding biologically active portions of an SES protein can be prepared by isolating a portion of one of the sequences in Appendix B, expressing the encoded portion of the SES protein or peptide (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the SES protein or peptide.

The invention further encompasses nucleic acid molecules that differ from one of the nucleotide sequences shown in Appendix A (and portions thereof) due to degeneracy of the genetic code and thus encode the same SES protein as that encoded by the nucleotide sequences shown in Appendix A. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in Appendix B. In a still further embodiment, the nucleic acid molecule of the invention encodes a full length *C. glutamicum* protein which is substantially homologous to an amino acid sequence of Appendix B (encoded by an open reading frame shown in Appendix A).

It will be understood by one of ordinary skill in the art that in one embodiment the sequences of the invention are not meant to include the sequences of the prior art, such as those Genbank sequences set forth in Tables 2 or 4 which were available prior to the present invention. In one embodiment, the invention includes nucleotide and amino acid sequences having a percent identity to a nucleotide or amino acid sequence of the invention which is greater than that of a sequence of the prior art (*e.g.*, a Genbank

sequence (or the protein encoded by such a sequence) set forth in Tables 2 or 4). For example, the invention includes a nucleotide sequence which is greater than and/or at least 71% identical to the nucleotide sequence designated RXA01278 (SEQ ID NO:1), a nucleotide sequence which is greater than and/or at least 38% identical to the nucleotide sequence designated RXA01020 (SEQ ID NO:25), and a nucleotide sequence which is greater than and/or at least 54% identical to the nucleotide sequence designated RXA02078 (SEQ ID NO:39). One of ordinary skill in the art would be able to calculate the lower threshold of percent identity for any given sequence of the invention by examining the GAP-calculated percent identity scores set forth in Table 4 for each of the three top hits for the given sequence, and by subtracting the highest GAP-calculated percent identity from 100 percent. One of ordinary skill in the art will also appreciate that nucleic acid and amino acid sequences having percent identities greater than the lower threshold so calculated (*e.g.*, at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%, preferably at least about 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more identical) are also encompassed by the invention.

In addition to the *C. glutamicum* SES nucleotide sequences shown in Appendix A, it will be appreciated by those of ordinary skill in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of SES proteins may exist within a population (*e.g.*, the *C. glutamicum* population). Such genetic polymorphism in the SES gene may exist among individuals within a population due to natural variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding an SES protein, preferably a *C. glutamicum* SES protein. Such natural variations can typically result in 1-5% variance in the nucleotide sequence of the SES gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in SES that are the result of natural variation and that do not alter the functional activity of SES proteins are intended to be within the scope of the invention.

Nucleic acid molecules corresponding to natural variants and non-*C. glutamicum* homologues of the *C. glutamicum* SES DNA of the invention can be isolated based on their homology to the *C. glutamicum* SES nucleic acid disclosed herein using the *C. glutamicum* DNA, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid

molecule comprising a nucleotide sequence of Appendix A. In other embodiments, the nucleic acid is at least 30, 50, 100, 250 or more nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 65%, more preferably at least about 70%, and even more preferably at least about 75% or more homologous to each other typically remain hybridized to each other. Such stringent conditions are known to those of ordinary skill in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to a sequence of Appendix A corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein). In one embodiment, the nucleic acid encodes a natural *C. glutamicum* SES protein.

In addition to naturally-occurring variants of the SES sequence that may exist in the population, one of ordinary skill in the art will further appreciate that changes can be introduced by mutation into a nucleotide sequence of Appendix A, thereby leading to changes in the amino acid sequence of the encoded SES protein, without altering the functional ability of the SES protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of Appendix A. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one of the SES proteins (Appendix B) without altering the activity of said SES protein, whereas an "essential" amino acid residue is required for SES protein activity. Other amino acid residues, however, (*e.g.*, those that are not conserved or only semi-conserved in the domain having SES activity) may not be essential for activity and thus are likely to be amenable to alteration without altering SES activity.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding SES proteins that contain changes in amino acid residues that are not essential for SES activity. Such SES proteins differ in amino acid sequence from a sequence contained in Appendix B yet retain at least one of the SES activities described herein. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about

50% homologous to an amino acid sequence of Appendix B and is capable of participating in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or has one or more activities set forth in Table 1. Preferably, the protein encoded by the nucleic acid molecule is at least about 50-60% homologous to one of the sequences in Appendix B, more preferably at least about 60-70% homologous to one of the sequences in Appendix B, even more preferably at least about 70-80%, 80-90%, 90-95% homologous to one of the sequences in Appendix B, and most preferably at least about 96%, 97%, 98%, or 99% homologous to one of the sequences in Appendix B.

To determine the percent homology of two amino acid sequences (*e.g.*, one of the sequences of Appendix B and a mutant form thereof) or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of one protein or nucleic acid for optimal alignment with the other protein or nucleic acid). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in one sequence (*e.g.*, one of the sequences of Appendix B) is occupied by the same amino acid residue or nucleotide as the corresponding position in the other sequence (*e.g.*, a mutant form of the sequence selected from Appendix B), then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % homology = # of identical positions/total # of positions x 100).

An isolated nucleic acid molecule encoding an SES protein homologous to a protein sequence of Appendix B can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of Appendix A such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into one of the sequences of Appendix A by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine,

09602839 .062300

proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an SES protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an SES coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for an SES activity described herein to identify mutants that retain SES activity. Following mutagenesis of one of the sequences of Appendix A, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using, for example, assays described herein (see Example 8 of the Exemplification).

In addition to the nucleic acid molecules encoding SES proteins described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded DNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire SES coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an SES protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the entire coding region of SEQ ID NO. 1 (RXA01278) comprises nucleotides 1 to 2127). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding SES. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding SES disclosed herein (*e.g.*, the sequences set forth in Appendix A), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of SES mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of SES mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SES mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in

the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an SES protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic promoter are preferred.

09622639.062300

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.*(1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.*(1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.*(1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave SES mRNA transcripts to thereby inhibit translation of SES mRNA. A ribozyme having specificity for an SES-encoding nucleic acid can be designed based upon the nucleotide sequence of an SES DNA disclosed herein (*i.e.*, SEQ ID NO. 1 (RXA01278 in Appendix A)). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an SES-encoding mRNA. See, *e.g.*, Cech *et al.*U.S. Patent No. 4,987,071 and Cech *et al.*U.S. Patent No. 5,116,742. Alternatively, SES mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, SES gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of an SES nucleotide sequence (*e.g.*, an SES promoter and/or enhancers) to form triple helical structures that prevent transcription of an SES gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.*(1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

B. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an SES protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of

autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which are operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells. Preferred regulatory sequences are, for example, promoters such as cos-, tac-, trp-, tet-, trp-tet-, lpp-, lac-, lpp-lac-, $lacI^q$-, T7-, T5-, T3-, gal-, trc-, ara-, SP6-, arny, SPO2, λ-P_R- or λ P_L, which are used preferably in bacteria. Additional regulatory sequences are, for example, promoters from yeasts and fungi, such as ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH, promoters from plants such as CaMV/35S, SSU, OCS, lib4, usp, STLS1, B33, nos or ubiquitin- or phaseolin-promoters. It is also possible to use artificial promoters. It will be appreciated by one of ordinary skill in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or

peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, SES proteins, mutant forms of SES proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of SES proteins in prokaryotic or eukaryotic cells. For example, SES genes can be expressed in bacterial cells such as *C. glutamicum*, insect cells (using baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A. *et al.*(1992) "Foreign gene expression in yeast: a review", *Yeast* 8: 423-488; van den Hondel, C.A.M.J.J. *et al.*(1991) "Heterologous gene expression in filamentous fungi" in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F. et al., eds., p. 1-28, Cambridge University Press: Cambridge), algae and multicellular plant cells (see Schmidt, R. and Willmitzer, L. (1988) High efficiency *Agrobacterium tumefaciens* –mediated transformation of *Arabidopsis thaliana* leaf and cotyledon explants" *Plant Cell Rep.*: 583-586), or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein but also to the C-terminus or fused within suitable regions in the proteins. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein. In one embodiment, the coding sequence of the SES protein is cloned into a pGEX expression vector to create a vector encoding a fusion protein comprising, from

the N-terminus to the C-terminus, GST-thrombin cleavage site-X protein. The fusion protein can be purified by affinity chromatography using glutathione-agarose resin. Recombinant SES protein unfused to GST can be recovered by cleavage of the fusion protein with thrombin.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) pLG338, pACYC184, pBR322, pUC18, pUC19, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11, pBdCl, and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89; and Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. For transformation of other varieties of bacteria, appropriate vectors may be selected. For example, the plasmids pIJ101, pIJ364, pIJ702 and pIJ361 are known to be useful in transforming Streptomyces, while plasmids pUB110, pC194, or pBD214 are suited for transformation of Bacillus species. Several plasmids of use in the transfer of genetic information into Corynebacterium include pHM1519, pBL1, pSA77, or pAJ667 (Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018).One strategy to maximize recombinant protein expression is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as *C. glutamicum* (Wada *et al.*(1992) *Nucleic Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the SES protein expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S cerevisiae* include pYepSec1 (Baldari, et al., (1987) *Embo J.* 6:229-234, 2 μ, pAG-1, Yep6, Yep13, pEMBLYe23, pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for the construction of vectors appropriate for use in other fungi, such as the

filamentous fungi, include those detailed in: van den Hondel, C.A.M.J.J. & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, J.F. Peberdy, et al., eds., p. 1-28, Cambridge University Press: Cambridge, and Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York (IBSN 0 444 904018).

Alternatively, the SES proteins of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.*(1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In another embodiment, the SES proteins of the invention may be expressed in unicellular plant cells (such as algae) or in plant cells from higher plants (*e.g.*, the spermatophytes, such as crop plants). Examples of plant expression vectors include those detailed in: Becker, D., Kemper, E., Schell, J. and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", *Plant Mol. Biol.* 20: 1195-1197; and Bevan, M.W. (1984) "Binary *Agrobacterium* vectors for plant transformation", *Nucl. Acid. Res.* 12: 8711-8721, and include pLGV23, pGHlac+, pBIN19, pAK2004, and pDH51 (Pouwels *et al.*, eds. (1985) Cloning Vectors. Elsevier: New York IBSN 0 444 904018).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.*(1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.*(1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto

and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.*(1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.*(1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to SES mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. et al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) (1986).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an SES protein can be expressed in bacterial cells such as *C. glutamicum*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to one of ordinary skill in the art. Microorganisms related

to *Corynebacterium glutamicum* which may be conveniently used as host cells for the nucleic acid and protein molecules of the invention are set forth in Table 3.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection", "conjugation" and "transduction" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, linear DNA or RNA (*e.g.*, a linearized vector or a gene construct alone without a vector) or nucleic acid in the form of a vector (*e.g.*, a plasmid, phage, phasmid, phagemid, transposon or other DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemical-mediated transfer, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.*(*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an SES protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by, for example, drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

To create a homologous recombinant microorganism, a vector is prepared which contains at least a portion of an SES gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the SES gene. Preferably, this SES gene is a *Corynebacterium glutamicum* SES gene, but it can be a homologue from a related bacterium or even from a mammalian, yeast, or insect source. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous SES gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous SES gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous SES protein). In the homologous recombination vector, the altered portion

of the SES gene is flanked at its 5' and 3' ends by additional nucleic acid of the SES gene to allow for homologous recombination to occur between the exogenous SES gene carried by the vector and an endogenous SES gene in a microorganism. The additional flanking SES nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see *e.g.*, Thomas, K.R., and Capecchi, M.R. (1987) Cell 51: 503 for a description of homologous recombination vectors). The vector is introduced into a microorganism (*e.g.*, by electroporation) and cells in which the introduced SES gene has homologously recombined with the endogenous SES gene are selected, using art-known techniques.

In another embodiment, recombinant microorganisms can be produced which contain selected systems which allow for regulated expression of the introduced gene. For example, inclusion of an SES gene on a vector placing it under control of the lac operon permits expression of the SES gene only in the presence of IPTG. Such regulatory systems are well known in the art.

In another embodiment, an endogenous SES gene in a host cell is disrupted (*e.g.*, by homologous recombination or other genetic means known in the art) such that expression of its protein product does not occur. In another embodiment, an endogenous or introduced SES gene in a host cell has been altered by one or more point mutations, deletions, or inversions, but still encodes a functional SES protein. In still another embodiment, one or more of the regulatory regions (*e.g.*, a promoter, repressor, or inducer) of an SES gene in a microorganism has been altered (*e.g.*, by deletion, truncation, inversion, or point mutation) such that the expression of the SES gene is modulated. One of ordinary skill in the art will appreciate that host cells containing more than one of the described SES gene and protein modifications may be readily produced using the methods of the invention, and are meant to be included in the present invention.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an SES protein. Accordingly, the invention further provides methods for producing SES proteins using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding an SES protein has been introduced, or into which genome has been introduced a gene encoding a wild-type or altered SES protein) in a suitable medium until SES protein is produced. In another embodiment, the method further comprises isolating SES proteins from the medium or the host cell.

C. Isolated SES Proteins

Another aspect of the invention pertains to isolated SES proteins, and biologically active portions thereof. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of SES protein in which the protein is separated from cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of SES protein having less than about 30% (by dry weight) of non-SES protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-SES protein, still more preferably less than about 10% of non-SES protein, and most preferably less than about 5% non-SES protein. When the SES protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of SES protein in which the protein is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of SES protein having less than about 30% (by dry weight) of chemical precursors or non-SES chemicals, more preferably less than about 20% chemical precursors or non-SES chemicals, still more preferably less than about 10% chemical precursors or non-SES chemicals, and most preferably less than about 5% chemical precursors or non-SES chemicals. In preferred embodiments, isolated proteins or biologically active portions thereof lack contaminating proteins from the same organism from which the SES protein is derived. Typically, such proteins are produced by recombinant expression of, for example, a *C. glutamicum* SES protein in a microorganism such as *C. glutamicum*.

An isolated SES protein or a portion thereof of the invention can participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or has one or more of the activities set forth in Table 1. In preferred embodiments, the protein or portion thereof comprises an amino acid sequence which is sufficiently homologous to an amino acid sequence of Appendix B such that the protein or portion thereof maintains the ability to participate in the repair or recombination of DNA, in the transposition of genetic material, in gene

expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*. The portion of the protein is preferably a biologically active portion as described herein. In another preferred embodiment, an SES protein of the invention has an amino acid sequence shown in Appendix B. In yet another preferred embodiment, the SES protein has an amino acid sequence which is encoded by a nucleotide sequence which hybridizes, *e.g.*, hybridizes under stringent conditions, to a nucleotide sequence of Appendix A. In still another preferred embodiment, the SES protein has an amino acid sequence which is encoded by a nucleotide sequence that is at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%, preferably at least about 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to one of the nucleic acid sequences of Appendix A, or a portion thereof. Ranges and identity values intermediate to the above-recited values, (*e.g.*, 70-90% identical or 80-95% identical) are also intended to be encompassed by the present invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. The preferred SES proteins of the present invention also preferably possess at least one of the SES activities described herein. For example, a preferred SES protein of the present invention includes an amino acid sequence encoded by a nucleotide sequence which hybridizes, *e.g.*, hybridizes under stringent conditions, to a nucleotide sequence of Appendix A, and which can participate in the repair or recombination of DNA, in the transposition of genetic material, in gene expression (*i.e.*, the processes of transcription or translation), in protein folding, or in protein secretion in *Corynebacterium glutamicum*, or which has one or more of the activities set forth in Table 1.

In other embodiments, the SES protein is substantially homologous to an amino acid sequence of Appendix B and retains the functional activity of the protein of one of the sequences of Appendix B yet differs in amino acid sequence due to natural variation or mutagenesis, as described in detail in subsection I above. Accordingly, in another embodiment, the SES protein is a protein which comprises an amino acid sequence which is at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%, preferably at least about 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, or 70%, more preferably at least about 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, or 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or 90%, or 91%, 92%, 93%, 94%, and even more preferably at least about 95%, 96%, 97%, 98%, 99% or more homologous to an entire amino acid sequence of Appendix B and which has at least one

of the SES activities described herein. Ranges and identity values intermediate to the above-recited values, (*e.g.*, 70-90% identical or 80-95% identical) are also intended to be encompassed by the present invention. For example, ranges of identity values using a combination of any of the above values recited as upper and/or lower limits are intended to be included. In another embodiment, the invention pertains to a full length *C. glutamicum* protein which is substantially homologous to an entire amino acid sequence of Appendix B.

Biologically active portions of an SES protein include peptides comprising amino acid sequences derived from the amino acid sequence of an SES protein, *e.g.*, the an amino acid sequence shown in Appendix B or the amino acid sequence of a protein homologous to an SES protein, which include fewer amino acids than a full length SES protein or the full length protein which is homologous to an SES protein, and exhibit at least one activity of an SES protein. Typically, biologically active portions (peptides, *e.g.*, peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of an SES protein. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically active portions of an SES protein include one or more selected domains/motifs or portions thereof having biological activity.

SES proteins are preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the protein is cloned into an expression vector (as described above), the expression vector is introduced into a host cell (as described above) and the SES protein is expressed in the host cell. The SES protein can then be isolated from the cells by an appropriate purification scheme using standard protein purification techniques. Alternative to recombinant expression, an SES protein, polypeptide, or peptide can be synthesized chemically using standard peptide synthesis techniques. Moreover, native SES protein can be isolated from cells (*e.g.*, endothelial cells), for example using an anti-SES antibody, which can be produced by standard techniques utilizing an SES protein or fragment thereof of this invention.

The invention also provides SES chimeric or fusion proteins. As used herein, an SES "chimeric protein" or "fusion protein" comprises an SES polypeptide operatively linked to a non-SES polypeptide. An "SES polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an SES protein, whereas a "non-SES polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the SES protein, *e.g.*, a protein which is different from the SES protein and which is derived from the same or a different

Docket No. BGI-127CP

organism. Within the fusion protein, the term "operatively linked" is intended to indicate that the SES polypeptide and the non-SES polypeptide are fused in-frame to each other. The non-SES polypeptide can be fused to the N-terminus or C-terminus of the SES polypeptide. For example, in one embodiment the fusion protein is a GST-SES fusion protein in which the SES sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant SES proteins. In another embodiment, the fusion protein is an SES protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of an SES protein can be increased through use of a heterologous signal sequence.

Preferably, an SES chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An SES-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the SES protein.

Homologues of the SES protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the SES protein. As used herein, the term "homologue" refers to a variant form of the SES protein which acts as an agonist or antagonist of the activity of the SES protein. An agonist of the SES protein can retain substantially the same, or a subset, of the biological activities of the SES protein. An antagonist of the SES protein can inhibit one or more of the activities of the naturally occurring form of the SES protein, by, for example, competitively binding to a downstream or upstream member of a biochemical cascade which includes the SES protein, by binding to a target molecule with which the SES protein interacts, such that no function interaction is possible, or by binding directly to the SES protein and inhibiting its normal activity.

00323629.050900

In an alternative embodiment, homologues of the SES protein can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the SES protein for SES protein agonist or antagonist activity. In one embodiment, a variegated library of SES variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of SES variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential SES sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of SES sequences therein. There are a variety of methods which can be used to produce libraries of potential SES homologues from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential SES sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.*(1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.*(1984) *Science* 198:1056; Ike *et al.*(1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of the SES protein coding can be used to generate a variegated population of SES fragments for screening and subsequent selection of homologues of an SES protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an SES coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the SES protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of SES homologues. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a

desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify SES homologues (Arkin and Yourvan (1992) *PNAS* *89*:7811-7815; Delgrave *et al.*(1993) *Protein Engineering* 6(3):327-331).

In another embodiment, cell based assays can be exploited to analyze a variegated SES library, using methods well known in the art.

D. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, fusion proteins, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *C. glutamicum* and related organisms; mapping of genomes of organisms related to *C. glutamicum*; identification and localization of *C. glutamicum* sequences of interest; evolutionary studies; determination of SES protein regions required for function; modulation of an SES protein activity; and modulation of cellular production of a desired compound, such as a fine chemical.

The SES nucleic acid molecules of the invention have a variety of uses. First, they may be used to identify an organism as being *Corynebacterium glutamicum* or a close relative thereof. Also, they may be used to identify the presence of *C. glutamicum* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *C. glutamicum* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *C. glutamicum* gene which is unique to this organism, one can ascertain whether this organism is present.

Although *Corynebacterium glutamicum* itself is nonpathogenic, it is related to pathogenic species, such as *Corynebacterium diphtheriae*. *Corynebacterium diphtheriae* is the causative agent of diphtheria, a rapidly developing, acute, febrile infection which involves both local and systemic pathology. In this disease, a local lesion develops in the upper respiratory tract and involves necrotic injury to epithelial cells; the bacilli secrete toxin which is disseminated through this lesion to distal susceptible tissues of the body. Degenerative changes brought about by the inhibition of protein synthesis in these tissues, which include heart, muscle, peripheral nerves, adrenals, kidneys, liver and spleen, result in the systemic pathology of the disease. Diphtheria continues to have high incidence in many parts of the world, including Africa, Asia, Eastern Europe and the independent states of the former Soviet Union. An ongoing epidemic of diphtheria in the latter two regions has resulted in at least 5,000 deaths since 1990.

In one embodiment, the invention provides a method of identifying the presence or activity of *Cornyebacterium diphtheriae* in a subject. This method includes detection of one or more of the nucleic acid or amino acid sequences of the invention (*e.g.*, the sequences set forth in Appendix A or Appendix B) in a subject, thereby detecting the presence or activity of *Corynebacterium diphtheriae* in the subject. *C. glutamicum* and *C. diphtheriae* are related bacteria, and many of the nucleic acid and protein molecules in C. glutamicum are homologous to C. diphtheriae nucleic acid and protein molecules, and can therefore be used to detect C. diphtheriae in a subject.

The nucleic acid and protein molecules of the invention may also serve as markers for specific regions of the genome. This has utility not only in the mapping of the genome, but also for functional studies of *C. glutamicum* proteins. For example, to identify the region of the genome to which a particular *C. glutamicum* DNA-binding protein binds, the *C. glutamicum* genome could be digested, and the fragments incubated with the DNA-binding protein. Those which bind the protein may be additionally probed with the nucleic acid molecules of the invention, preferably with readily detectable labels; binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map of *C. glutamicum*, and, when performed multiple times with different enzymes, facilitates a rapid determination of the nucleic acid sequence to which the protein binds. Further, the nucleic acid molecules of the invention may be sufficiently homologous to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related bacteria, such as *Brevibacterium lactofermentum*.

The SES nucleic acid molecules of the invention are also useful for evolutionary and protein structural studies. The metabolic and transport processes in which the molecules of the invention participate are utilized by a wide variety of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and which are not, which may aid in determining those regions of the protein which are essential for the functioning of the enzyme. This type of determination is of value for protein engineering studies and may give an indication of what the protein can tolerate in terms of mutagenesis without losing function.

Manipulation of the SES nucleic acid molecules of the invention may result in the production of SES proteins having functional differences from the wild-type SES proteins. These proteins may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

09502839.052300

The invention provides methods for screening molecules which modulate the activity of an SES protein, either by interacting with the protein itself or a substrate or binding partner of the SES protein, or by modulating the transcription or translation of an SES nucleic acid molecule of the invention. In such methods, a microorganism expressing one or more SES proteins of the invention is contacted with one or more test compounds, and the effect of each test compound on the activity or level of expression of the SES protein is assessed.

The modulation of activity of proteins involved in *C. glutamicum* DNA repair, recombination, or transposition should impact the genetic stability of the cell. For example, by decreasing the number or activity of proteins involved in DNA repair mechanisms, one may decrease the ability of the cell to correct genetic errors, which should permit the simplified introduction of desired mutations into the genome (such as those encoding proteins involved in fine chemical production). Increasing the activity or number of transposons should result in a similarly increased mutation rate in the genome, and can permit facile duplication of desired genes (*e.g.*, those encoding fine chemical biosynthetic proteins) or disruption of undesired genes (*e.g.*, those encoding fine chemical degradation proteins). Conversely, by decreasing the number or activity of transposons or by increasing the number or activity of DNA repair proteins, it may be possible to increase the genetic stability of *C. glutamicum*, which in turn should result in better retention of introduced mutations in this microorganism through multiple generations in culture. Ideally, during mutagenesis and strain construction, one or more DNA repair systems would be decreased in activity and one or more transposons may be increased in activity, but once the desired mutation had been achieved in a strain, these the reverse would occur. Such manipulation is possible by placement of one or more DNA repair genes or transposons under control of an inducible repressor.

Modulation of proteins involved in transcription and translation in *C. glutamicum* can have both direct and indirect effects on the production of a fine chemical from these microorganisms. For example, by manipulating a protein which directly translates a gene (*e.g.*, a polymerase) or which directly regulates transcription (*e.g.*, a repressor or activator protein), it is possible to directly affect the expression of the target gene. In the case of genes encoding a protein involved in the biosynthesis or degradation of a fine chemical, this type of genetic manipulation should have a direct effect on the production of this fine chemical. Mutagenesis of a repressor protein such that it can no longer repress its target gene, or mutagenesis of an activator protein such that it is optimized in activity should lead to an increase in transcription of the target gene. If the target gene is, for example, a fine chemical biosynthetic gene, then an increase in production of that chemical may result, due to the overall greater number of

0050209 - 050209

transcripts present for the gene, which should result in greater numbers of the protein as well. Increasing the number or activity of a repressor protein for a target sequence or decreasing the number or activity of an activator protein for a target sequence when this sequence is, for example, a fine chemical degradative protein, then a similar increase in production of the fine chemical should result.

Indirect effects on fine chemical production may also arise due to manipulation of proteins involved in transcription and translation. For example, by modulating the activity or number of transcription factors (*e.g.*, the sigma factors) or translational repressors/activators which globally regulate transcription in *C. glutamicum* in response to environmental or metabolic factors, it should be possible to uncouple cellular transcription from environmental or metabolic regulation. In turn, this may permit continued transcription under conditions which would normally slow or altogether stop gene expression, such as those unfavorable conditions (*e.g.*, high temperature, low oxygen, high waste product levels) which exist in large-scale fermentor cultures. By increasing the rate of gene (*e.g.*, fine chemical biosynthetic gene) expression in such situations, the overall rate of fine product production may also be increased, at least due to the relatively greater number of fine chemical biosynthetic proteins in the cell. Principles and examples for modification of transcription and transcriptional regulation are described in, *e.g.*, Lewin, B. (1990) Genes IV, Part 3: "Controlling procaryotic genes by transcription" Oxford Univ. Press: Oxford, p. 213-301.

Modulation of the activity or number of proteins involved in polypeptide folding (*e.g.*, chaperones) may permit an increase in the overall production of correctly folded molecules in the cell. This has two effects: first, an overall increase in the number of proteins in the cell, due to the fact that fewer proteins are misfolded and degraded, and second, an increase in the number of any given protein that is correctly folded and thus active (see, *e.g.*, Thomas, J.G., Baneyx, F. (1997) *Protein Expression and Purification* 11(3): 289-296; Luo, Z.H., and Hua, Z.C. (1998) *Biochemistry and Molecular Biology International* 46(3): 471-477; Dale, G.E., *et al.*(1994) *Protein Engineering* 7(7): 925-931; Amrein, K.E. *et al.*(1995) *Proc. Natl. Acad. Sci. U.S.A.* 92(4): 1048-1052; and Caspers, P. *et al.*(1994) *Cell. Mol. Biol.* 40(5): 635-644). While such mutations result in an increase in the number of active proteins of all kinds, when coupled with additional mutations increasing the activity or number of, *e.g.*, a fine chemical biosynthetic protein, an additive effect in the amount of correctly folded, active desired protein may be obtained.

Manipulation of proteins involved in secretion of polypeptides from *C. glutamicum* such that they are improved in activity or number may directly improve the secretion of a proteinaceous fine chemical (*e.g.*, an enzyme) from this microorganism. It

is significantly easier to harvest and purify fine chemicals when they are secreted into the medium of large-scale cultures than when they are retained in the cell, so the yield and production of a fine chemical should be increased through such secretion system engineering. Genetic manipulation of these secretion proteins may also result in indirect improvements in the production of one or more fine chemicals. First, increased or decreased activity of one or more *C. glutamicum* secretion systems (as brought about by mutagenesis of one or more SES proteins involved in such pathways) may result in increased or decreased global secretion rates from the cell. Many such secreted proteins have functions critical for cell viability (*e.g.*, cell surface proteases or receptors). An alteration of a secretory pathway such that these proteins are more readily transported to their extracellular location may improve the overall viability of the cell, and thus result in greater numbers of *C. glutamicum* cells capable of producing fine chemicals during large-scale culture. Second, certain bacterial secretion systems, (*e.g.*, the sec system) are known to play a significant role in the process by which integral membrane proteins (*e.g.* channels, pores, or transporters) insert into cellular membranes. If the activity of one or more secretory pathway proteins is increased, then the ability of the cell to produce fine chemicals may be similarly increased, due to the presence of increased intracellular nutrient levels or decreased intracellular waste levels. If the activity of one or more such secretory pathway protein is decreased, then there may be insufficient nutrients available to support overproduction of desired compounds, or waste products may interfere with the biosynthesis of desired fine chemicals.

The aforementioned mutagenesis strategies for SES proteins to result in increased yields of a fine chemical from *C. glutamicum* are not meant to be limiting; variations on these strategies will be readily apparent to one of ordinary skill in the art. Using such strategies, and incorporating the mechanisms disclosed herein, the nucleic acid and protein molecules of the invention may be utilized to generate *C. glutamicum* or related strains of bacteria expressing mutated SES nucleic acid and protein molecules such that the yield, production, and/or efficiency of production of a desired compound is improved. This desired compound may be any product produced by *C. glutamicum*, which includes the final products of biosynthesis pathways and intermediates of naturally-occurring metabolic pathways, as well as molecules which do not naturally occur in the metabolism of *C. glutamicum*, but which are produced by a *C. glutamicum* strain of the invention.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patent applications, patents,

published patent applications, Tables, Appendices, and the sequence listing cited throughout this application are hereby incorporated by reference.

Exemplification

Example 1: Preparation of total genomic DNA of *Corynebacterium glutamicum* ATCC 13032

A culture of *Corynebacterium glutamicum* (ATCC 13032) was grown overnight at 30°C with vigorous shaking in BHI medium (Difco). The cells were harvested by centrifugation, the supernatant was discarded and the cells were resuspended in 5 ml buffer-I (5% of the original volume of the culture — all indicated volumes have been calculated for 100 ml of culture volume). Composition of buffer-I: 140.34 g/l sucrose, 2.46 g/l $MgSO_4 \times 7H_2O$, 10 ml/l KH_2PO_4 solution (100 g/l, adjusted to pH 6.7 with KOH), 50 ml/l M12 concentrate (10 g/l $(NH_4)_2SO_4$, 1 g/l NaCl, 2 g/l $MgSO_4 \times 7H_2O$, 0.2 g/l $CaCl_2$, 0.5 g/l yeast extract (Difco), 10 ml/l trace-elements-mix (200 mg/l $FeSO_4 \times H_2O$, 10 mg/l $ZnSO_4 \times 7\ H_2O$, 3 mg/l $MnCl_2 \times 4\ H_2O$, 30 mg/l H_3BO_3 20 mg/l $CoCl_2 \times 6\ H_2O$, 1 mg/l $NiCl_2 \times 6\ H_2O$, 3 mg/l $Na_2MoO_4 \times 2\ H_2O$, 500 mg/l complexing agent (EDTA or critic acid), 100 ml/l vitamins-mix (0.2 mg/l biotin, 0.2 mg/l folic acid, 20 mg/l p-amino benzoic acid, 20 mg/l riboflavin, 40 mg/l ca-panthothenate, 140 mg/l nicotinic acid, 40 mg/l pyridoxole hydrochloride, 200 mg/l myo-inositol). Lysozyme was added to the suspension to a final concentration of 2.5 mg/ml. After an approximately 4 h incubation at 37°C, the cell wall was degraded and the resulting protoplasts are harvested by centrifugation. The pellet was washed once with 5 ml buffer-I and once with 5 ml TE-buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). The pellet was resuspended in 4 ml TE-buffer and 0.5 ml SDS solution (10%) and 0.5 ml NaCl solution (5 M) are added. After adding of proteinase K to a final concentration of 200 µg/ml, the suspension is incubated for ca.18 h at 37°C. The DNA was purified by extraction with phenol, phenol-chloroform-isoamylalcohol and chloroform-isoamylalcohol using standard procedures. Then, the DNA was precipitated by adding 1/50 volume of 3 M sodium acetate and 2 volumes of ethanol, followed by a 30 min incubation at -20°C and a 30 min centrifugation at 12,000 rpm in a high speed centrifuge using a SS34 rotor (Sorvall). The DNA was dissolved in 1 ml TE-buffer containing 20 µg/ml RNaseA and dialysed at 4°C against 1000 ml TE-buffer for at least 3 hours. During this time, the buffer was exchanged 3 times. To aliquots of 0.4 ml of the dialysed DNA solution, 0.4 ml of 2 M LiCl and 0.8 ml of ethanol are added. After a 30 min incubation at -20°C, the DNA was collected by centrifugation (13,000 rpm, Biofuge Fresco, Heraeus, Hanau, Germany). The DNA pellet was dissolved in TE-buffer. DNA prepared by this procedure could be used for all purposes, including southern blotting or construction of genomic libraries.

Example 2: Construction of genomic libraries in *Escherichia coli* of *Corynebacterium glutamicum* ATCC13032.

Using DNA prepared as described in Example 1, cosmid and plasmid libraries were constructed according to known and well established methods (*see e.g.,* Sambrook, J. *et al.*(1989) "Molecular Cloning : A Laboratory Manual", Cold Spring Harbor Laboratory Press, or Ausubel, F.M. *et al.*(1994) "Current Protocols in Molecular Biology", John Wiley & Sons.)

Any plasmid or cosmid could be used. Of particular use were the plasmids pBR322 (Sutcliffe, J.G. (1979) *Proc. Natl. Acad. Sci. USA*, 75:3737-3741); pACYC177 (Change & Cohen (1978) *J. Bacteriol* 134:1141-1156), plasmids of the pBS series (pBSSK+, pBSSK- and others; Stratagene, LaJolla, USA), or cosmids as SuperCos1 (Stratagene, LaJolla, USA) or Lorist6 (Gibson, T.J., Rosenthal A. and Waterson, R.H. (1987) *Gene* 53:283-286. Gene libraries specifically for use in *C. glutamicum* may be constructed using plasmid pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol. Biotechnol.* 4: 256-263).

Example 3: DNA Sequencing and Computational Functional Analysis

Genomic libraries as described in Example 2 were used for DNA sequencing according to standard methods, in particular by the chain termination method using ABI377 sequencing machines (see *e.g.*, Fleischman, R.D. *et al.*(1995) "Whole-genome Random Sequencing and Assembly of Haemophilus Influenzae Rd., *Science*, 269:496-512). Sequencing primers with the following nucleotide sequences were used: 5'-GGAAACAGTATGACCATG-3' (SEQ ID NO: 617) or 5'-GTAAAACGACGGCCAGT-3' (SEQ ID NO: 618)

Example 4: *In vivo* Mutagenesis

In vivo mutagenesis of *Corynebacterium glutamicum* can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (*e.g. Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae*) which are impaired in their capabilities to maintain the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (*e.g.*, mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D. (1996) DNA repair mechanisms, in: *Escherichia coli* and *Salmonella*, p. 2277-2294, ASM: Washington.) Such strains are well known to one of ordinary skill in the art. The use of such strains is illustrated, for example, in Greener, A. and Callahan, M. (1994) *Strategies* 7: 32-34.

Example 5: DNA Transfer Between *Escherichia coli* and *Corynebacterium glutamicum*

Several *Corynebacterium* and *Brevibacterium* species contain endogenous plasmids (as *e.g.*, pHM1519 or pBL1) which replicate autonomously (for review see, *e.g.*,

Martin, J.F. *et al.*(1987) *Biotechnology*, 5:137-146). Shuttle vectors for *Escherichia coli* and *Corynebacterium glutamicum* can be readily constructed by using standard vectors for *E. coli* (Sambrook, J. *et al.*(1989), "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press or Ausubel, F.M. *et al.*(1994) "Current Protocols in Molecular Biology", John Wiley & Sons) to which a origin or replication for and a suitable marker from *Corynebacterium glutamicum* is added. Such origins of replication are preferably taken from endogenous plasmids isolated from *Corynebacterium* and *Brevibacterium* species. Of particular use as transformation markers for these species are genes for kanamycin resistance (such as those derived from the Tn5 or Tn903 transposons) or chloramphenicol (Winnacker, E.L. (1987) "From Genes to Clones — Introduction to Gene Technology, VCH, Weinheim). There are numerous examples in the literature of the construction of a wide variety of shuttle vectors which replicate in both *E. coli and C. glutamicum*, and which can be used for several purposes, including gene over-expression (for reference, see *e.g.*, Yoshihama, M. *et al.*(1985) *J. Bacteriol.* 162:591-597, Martin J.F. *et al.*(1987) *Biotechnology*, 5:137-146 and Eikmanns, B.J. *et al.*(1991) *Gene*, 102:93-98).

Using standard methods, it is possible to clone a gene of interest into one of the shuttle vectors described above and to introduce such a hybrid vectors into strains of *Corynebacterium glutamicum*. Transformation of *C. glutamicum* can be achieved by protoplast transformation (Kastsumata, R. *et al.*(1984) *J. Bacteriol.* 159306-311), electroporation (Liebl, E. *et al.*(1989) *FEMS Microbiol. Letters*, 53:399-303) and in cases where special vectors are used, also by conjugation (as described *e.g.* in Schäfer, A *et al.*(1990) *J. Bacteriol.* 172:1663-1666). It is also possible to transfer the shuttle vectors for *C. glutamicum* to *E. coli* by preparing plasmid DNA from *C. glutamicum* (using standard methods well-known in the art) and transforming it into *E. coli*. This transformation step can be performed using standard methods, but it is advantageous to use an Mcr-deficient *E. coli* strain, such as NM522 (Gough & Murray (1983) *J. Mol. Biol.* 166:1-19).

Genes may be overexpressed in *C. glutamicum* strains using plasmids which comprise pCG1 (U.S. Patent No. 4,617,267) or fragments thereof, and optionally the gene for kanamycin resistance from TN903 (Grindley, N.D. and Joyce, C.M. (1980) *Proc. Natl. Acad. Sci. USA* 77(12): 7176-7180). In addition, genes may be overexpressed in *C. glutamicum* strains using plasmid pSL109 (Lee, H.-S. and A. J. Sinskey (1994) *J. Microbiol. Biotechnol.* 4: 256-263).

Aside from the use of replicative plasmids, gene overexpression can also be achieved by integration into the genome. Genomic integration in *C. glutamicum* or other Corynebacterium or Brevibacterium species may be accomplished by well-known

methods, such as homologous recombination with genomic region(s), restriction endonuclease mediated integration (REMI) (see, *e.g.*, DE Patent 19823834), or through the use of transposons. It is also possible to modulate the activity of a gene of interest by modifying the regulatory regions (*e.g.*, a promoter, a repressor, and/or an enhancer) by sequence modification, insertion, or deletion using site-directed methods (such as homologous recombination) or methods based on random events (such as transposon mutagenesis or REMI). Nucleic acid sequences which function as transcriptional terminators may also be inserted 3' to the coding region of one or more genes of the invention; such terminators are well-known in the art and are described, for example, in Winnacker, E.L. (1987) From Genes to Clones – Introduction to Gene Technology. VCH: Weinheim.

Example 6: Assessment of the Expression of the Mutant Protein

Observations of the activity of a mutated protein in a transformed host cell rely on the fact that the mutant protein is expressed in a similar fashion and in a similar quantity to that of the wild-type protein. A useful method to ascertain the level of transcription of the mutant gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot (for reference see, for example, Ausubel *et al.*(1988) Current Protocols in Molecular Biology, Wiley: New York), in which a primer designed to bind to the gene of interest is labeled with a detectable tag (usually radioactive or chemiluminescent), such that when the total RNA of a culture of the organism is extracted, run on gel, transferred to a stable matrix and incubated with this probe, the binding and quantity of binding of the probe indicates the presence and also the quantity of mRNA for this gene. This information is evidence of the degree of transcription of the mutant gene. Total cellular RNA can be prepared from *Corynebacterium glutamicum* by several methods, all well-known in the art, such as that described in Bormann, E.R. *et al.*(1992) *Mol. Microbiol.* 6: 317-326.

To assess the presence or relative quantity of protein translated from this mRNA, standard techniques, such as a Western blot, may be employed (see, for example, Ausubel *et al.*(1988) Current Protocols in Molecular Biology, Wiley: New York). In this process, total cellular proteins are extracted, separated by gel electrophoresis, transferred to a matrix such as nitrocellulose, and incubated with a probe, such as an antibody, which specifically binds to the desired protein. This probe is generally tagged with a chemiluminescent or colorimetric label which may be readily detected. The presence and quantity of label observed indicates the presence and quantity of the desired mutant protein present in the cell.

00320956 . 032801

Example 7: Growth of Genetically Modified *Corynebacterium glutamicum* — Media and Culture Conditions

Genetically modified *Corynebacteria* are cultured in synthetic or natural growth media. A number of different growth media for Corynebacteria are both well-known and readily available (Lieb *et al.*(1989) *Appl. Microbiol. Biotechnol.*, 32:205-210; von der Osten *et al.*(1998) *Biotechnology Letters*, 11:11-16; Patent DE 4,120,867; Liebl (1992) "The Genus *Corynebacterium*, in: The Procaryotes, Volume II, Balows, A. *et al.*, eds. Springer-Verlag). These media consist of one or more carbon sources, nitrogen sources, inorganic salts, vitamins and trace elements. Preferred carbon sources are sugars, such as mono-, di-, or polysaccharides. For example, glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose serve as very good carbon sources. It is also possible to supply sugar to the media via complex compounds such as molasses or other by-products from sugar refinement. It can also be advantageous to supply mixtures of different carbon sources. Other possible carbon sources are alcohols and organic acids, such as methanol, ethanol, acetic acid or lactic acid. Nitrogen sources are usually organic or inorganic nitrogen compounds, or materials which contain these compounds. Exemplary nitrogen sources include ammonia gas or ammonia salts, such as NH_4Cl or $(NH_4)_2SO_4$, NH_4OH, nitrates, urea, amino acids or complex nitrogen sources like corn steep liquor, soy bean flour, soy bean protein, yeast extract, meat extract and others.

Inorganic salt compounds which may be included in the media include the chloride-, phosphorous- or sulfate- salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron. Chelating compounds can be added to the medium to keep the metal ions in solution. Particularly useful chelating compounds include dihydroxyphenols, like catechol or protocatechuate, or organic acids, such as citric acid. It is typical for the media to also contain other growth factors, such as vitamins or growth promoters, examples of which include biotin, riboflavin, thiamin, folic acid, nicotinic acid, pantothenate and pyridoxin. Growth factors and salts frequently originate from complex media components such as yeast extract, molasses, corn steep liquor and others. The exact composition of the media compounds depends strongly on the immediate experiment and is individually decided for each specific case. Information about media optimization is available in the textbook "Applied Microbiol. Physiology, A Practical Approach (*eds.* P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). It is also possible to select growth media from commercial suppliers, like standard 1 (Merck) or BHI (grain heart infusion, DIFCO) or others.

All medium components are sterilized, either by heat (20 minutes at 1.5 bar and 121°C) or by sterile filtration. The components can either be sterilized together or, if

necessary, separately. All media components can be present at the beginning of growth, or they can optionally be added continuously or batchwise.

Culture conditions are defined separately for each experiment. The temperature should be in a range between 15°C and 45°C. The temperature can be kept constant or can be altered during the experiment. The pH of the medium should be in the range of 5 to 8.5, preferably around 7.0, and can be maintained by the addition of buffers to the media. An exemplary buffer for this purpose is a potassium phosphate buffer. Synthetic buffers such as MOPS, HEPES, ACES and others can alternatively or simultaneously be used. It is also possible to maintain a constant culture pH through the addition of NaOH or NH_4OH during growth. If complex medium components such as yeast extract are utilized, the necessity for additional buffers may be reduced, due to the fact that many complex compounds have high buffer capacities. If a fermentor is utilized for culturing the micro-organisms, the pH can also be controlled using gaseous ammonia.

The incubation time is usually in a range from several hours to several days. This time is selected in order to permit the maximal amount of product to accumulate in the broth. The disclosed growth experiments can be carried out in a variety of vessels, such as microtiter plates, glass tubes, glass flasks or glass or metal fermentors of different sizes. For screening a large number of clones, the microorganisms should be cultured in microtiter plates, glass tubes or shake flasks, either with or without baffles. Preferably 100 ml shake flasks are used, filled with 10% (by volume) of the required growth medium. The flasks should be shaken on a rotary shaker (amplitude 25 mm) using a speed-range of 100 – 300 rpm. Evaporation losses can be diminished by the maintenance of a humid atmosphere; alternatively, a mathematical correction for evaporation losses should be performed.

If genetically modified clones are tested, an unmodified control clone or a control clone containing the basic plasmid without any insert should also be tested. The medium is inoculated to an OD_{600} of O.5 – 1.5 using cells grown on agar plates, such as CM plates (10 g/l glucose, 2,5 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l NaCl, 2 g/l urea, 10 g/l polypeptone, 5 g/l yeast extract, 5 g/l meat extract, 22 g/l agar, pH 6.8 with 2M NaOH) that had been incubated at 30°C. Inoculation of the media is accomplished by either introduction of a saline suspension of *C. glutamicum* cells from CM plates or addition of a liquid preculture of this bacterium.

Example 8 – *In vitro* Analysis of the Function of Mutant Proteins

The determination of activities and kinetic parameters of enzymes is well established in the art. Experiments to determine the activity of any given altered enzyme must be tailored to the specific activity of the wild-type enzyme, which is well

within the ability of one of ordinary skill in the art. Overviews about enzymes in general, as well as specific details concerning structure, kinetics, principles, methods, applications and examples for the determination of many enzyme activities may be found, for example, in the following references: Dixon, M., and Webb, E.C., (1979) Enzymes. Longmans: London; Fersht, (1985) Enzyme Structure and Mechanism. Freeman: New York; Walsh, (1979) Enzymatic Reaction Mechanisms. Freeman: San Francisco; Price, N.C., Stevens, L. (1982) Fundamentals of Enzymology. Oxford Univ. Press: Oxford; Boyer, P.D., ed. (1983) The Enzymes, 3rd ed. Academic Press: New York; Bisswanger, H., (1994) Enzymkinetik, 2nd ed. VCH: Weinheim (ISBN 3527300325); Bergmeyer, H.U., Bergmeyer, J., Graßl, M., eds. (1983-1986) Methods of Enzymatic Analysis, 3rd ed., vol. I-XII, Verlag Chemie: Weinheim; and Ullmann's Encyclopedia of Industrial Chemistry (1987) vol. A9, "Enzymes". VCH: Weinheim, p. 352-363.

The activity of proteins which bind to DNA can be measured by several well-established methods, such as DNA band-shift assays (also called gel retardation assays). The effect of such proteins on the expression of other molecules can be measured using reporter gene assays (such as that described in Kolmar, H. *et al.*(1995) *EMBO J.* 14: 3895-3904 and references cited therein). Reporter gene test systems are well known and established for applications in both pro- and eukaryotic cells, using enzymes such as beta-galactosidase, green fluorescent protein, and several others.

The determination of activity of membrane-transport proteins can be performed according to techniques such as those described in Gennis, R.B. (1989) "Pores, Channels and Transporters", in Biomembranes, Molecular Structure and Function, Springer: Heidelberg, p. 85-137; 199-234; and 270-322.

Example 9: Analysis of Impact of Mutant Protein on the Production of the Desired Product

The effect of the genetic modification in *C. glutamicum* on production of a desired compound (such as an amino acid) can be assessed by growing the modified microorganism under suitable conditions (such as those described above) and analyzing the medium and/or the cellular component for increased production of the desired product (*i.e.*, an amino acid). Such analysis techniques are well known to one of ordinary skill in the art, and include spectroscopy, thin layer chromatography, staining methods of various kinds, enzymatic and microbiological methods, and analytical chromatography such as high performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A. et al., (1987) "Applications of HPLC in Biochemistry" in:

Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm *et al.*(1993) Biotechnology, vol. 3, Chapter III: "Product recovery and purification", page 469-714, VCH: Weinheim; Belter, P.A. *et al.*(1988) Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S. (1992) Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D. (1988) Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.)

In addition to the measurement of the final product of fermentation, it is also possible to analyze other components of the metabolic pathways utilized for the production of the desired compound, such as intermediates and side-products, to determine the overall efficiency of production of the compound. Analysis methods include measurements of nutrient levels in the medium (*e.g.*, sugars, hydrocarbons, nitrogen sources, phosphate, and other ions), measurements of biomass composition and growth, analysis of the production of common metabolites of biosynthetic pathways, and measurement of gasses produced during fermentation. Standard methods for these measurements are outlined in Applied Microbial Physiology, A Practical Approach, P.M. Rhodes and P.F. Stanbury, eds., IRL Press, p. 103-129; 131-163; and 165-192 (ISBN: 0199635773) and references cited therein.

Example 10: Purification of the Desired Product from *C. glutamicum* Culture

Recovery of the desired product from the *C. glutamicum* cells or supernatant of the above-described culture can be performed by various methods well known in the art. If the desired product is not secreted from the cells, the cells can be harvested from the culture by low-speed centrifugation, the cells can be lysed by standard techniques, such as mechanical force or sonication. The cellular debris is removed by centrifugation, and the supernatant fraction containing the soluble proteins is retained for further purification of the desired compound. If the product is secreted from the *C. glutamicum* cells, then the cells are removed from the culture by low-speed centrifugation, and the supernate fraction is retained for further purification.

The supernatant fraction from either purification method is subjected to chromatography with a suitable resin, in which the desired molecule is either retained on a chromatography resin while many of the impurities in the sample are not, or where the impurities are retained by the resin while the sample is not. Such chromatography steps may be repeated as necessary, using the same or different chromatography resins. One of ordinary skill in the art would be well-versed in the selection of appropriate

chromatography resins and in their most efficacious application for a particular molecule to be purified. The purified product may be concentrated by filtration or ultrafiltration, and stored at a temperature at which the stability of the product is maximized.

There are a wide array of purification methods known to the art and the preceding method of purification is not meant to be limiting. Such purification techniques are described, for example, in Bailey, J.E. & Ollis, D.F. Biochemical Engineering Fundamentals, McGraw-Hill: New York (1986).

The identity and purity of the isolated compounds may be assessed by techniques standard in the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin layer chromatography, NIRS, enzymatic assay, or microbiologically. Such analysis methods are reviewed in: Patek *et al.*(1994) *Appl. Environ. Microbiol.* 60: 133-140; Malakhova *et al.*(1996) *Biotekhnologiya* 11: 27-32; and Schmidt *et al.*(1998) *Bioprocess Engineer.* 19: 67-70. Ulmann's Encyclopedia of Industrial Chemistry, (1996) vol. A27, VCH: Weinheim, p. 89-90, p. 521-540, p. 540-547, p. 559-566, 575-581 and p. 581-587; Michal, G. (1999) Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A. *et al.*(1987) Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17.

Example 11: Analysis of the Gene Sequences of the Invention

The comparison of sequences and determination of percent homology between two sequences are art-known techniques, and can be accomplished using a mathematical algorithm, such as the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci.* USA 87:2264-68, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.*(1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to SES nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to SES protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, one of ordinary skill in the art will know how to optimize the parameters of the program (*e.g.*, XBLAST and NBLAST) for the specific sequence being analyzed.

Another example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Meyers and Miller ((1988) *Comput. Appl. Biosci.* 4: 11-17). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art, and include ADVANCE and ADAM. described in Torelli and Robotti (1994) *Comput. Appl. Biosci.* 10:3-5; and FASTA, described in Pearson and Lipman (1988) *P.N.A.S.* 85:2444-8.

The percent homology between two amino acid sequences can also be accomplished using the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. The percent homology between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package, using standard parameters, such as a gap weight of 50 and a length weight of 3.

A comparative analysis of the gene sequences of the invention with those present in Genbank has been performed using techniques known in the art (see, *e.g.*, Bexevanis and Ouellette, eds. (1998) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins. John Wiley and Sons: New York). The gene sequences of the invention were compared to genes present in Genbank in a three-step process. In a first step, a BLASTN analysis (*e.g.*, a local alignment analysis) was performed for each of the sequences of the invention against the nucleotide sequences present in Genbank, and the top 500 hits were retained for further analysis. A subsequent FASTA search (*e.g.*, a combined local and global alignment analysis, in which limited regions of the sequences are aligned) was performed on these 500 hits. Each gene sequence of the invention was subsequently globally aligned to each of the top three FASTA hits, using the GAP program in the GCG software package (using standard parameters). In order to obtain correct results, the length of the sequences extracted from Genbank were adjusted to the length of the query sequences by methods well-known in the art. The results of this analysis are set forth in Table 4. The resulting data is identical to that which would have been obtained had a GAP (global) analysis alone been performed on each of the genes of the invention in comparison with each of the references in Genbank, but required significantly reduced computational time as compared to such a database-wide GAP (global) analysis. Sequences of the invention for which no alignments above the cutoff values were obtained are indicated on Table 4 by the absence of alignment information. It will further be understood by one of ordinary skill in the art that the GAP alignment

homology percentages set forth in Table 4 under the heading "% homology (GAP)" are listed in the European numerical format, wherein a ',' represents a decimal point. For example, a value of "40,345" in this column represents "40.345%".

Example 12: Construction and Operation of DNA Microarrays

The sequences of the invention may additionally be used in the construction and application of DNA microarrays (the design, methodology, and uses of DNA arrays are well known in the art, and are described, for example, in Schena, M. *et al.*(1995) *Science* 270: 467-470; Wodicka, L. *et al.*(1997) *Nature Biotechnology* 15: 1359-1367; DeSaizieu, A. *et al.*(1998) *Nature Biotechnology* 16: 45-48; and DeRisi, J.L. *et al.*(1997) *Science* 278: 680-686).

DNA microarrays are solid or flexible supports consisting of nitrocellulose, nylon, glass, silicone, or other materials. Nucleic acid molecules may be attached to the surface in an ordered manner. After appropriate labeling, other nucleic acids or nucleic acid mixtures can be hybridized to the immobilized nucleic acid molecules, and the label may be used to monitor and measure the individual signal intensities of the hybridized molecules at defined regions. This methodology allows the simultaneous quantification of the relative or absolute amount of all or selected nucleic acids in the applied nucleic acid sample or mixture. DNA microarrays, therefore, permit an analysis of the expression of multiple (as many as 6800 or more) nucleic acids in parallel (see, *e.g.*, Schena, M. (1996) *BioEssays* 18(5): 427-431).

The sequences of the invention may be used to design oligonucleotide primers which are able to amplify defined regions of one or more *C. glutamicum* genes by a nucleic acid amplification reaction such as the polymerase chain reaction. The choice and design of the 5' or 3' oligonucleotide primers or of appropriate linkers allows the covalent attachment of the resulting PCR products to the surface of a support medium described above (and also described, for example, Schena, M. *et al.*(1995) *Science* 270: 467-470).

Nucleic acid microarrays may also be constructed by *in situ* oligonucleotide synthesis as described by Wodicka, L. *et al.*(1997) *Nature Biotechnology* 15: 1359-1367. By photolithographic methods, precisely defined regions of the matrix are exposed to light. Protective groups which are photolabile are thereby activated and undergo nucleotide addition, whereas regions that are masked from light do not undergo any modification. Subsequent cycles of protection and light activation permit the synthesis of different oligonucleotides at defined positions. Small, defined regions of the genes of the invention may be synthesized on microarrays by solid phase oligonucleotide synthesis.

09602839.062300

The nucleic acid molecules of the invention present in a sample or mixture of nucleotides may be hybridized to the microarrays. These nucleic acid molecules can be labeled according to standard methods. In brief, nucleic acid molecules (*e.g.*, mRNA molecules or DNA molecules) are labeled by the incorporation of isotopically or fluorescently labeled nucleotides, *e.g.*, during reverse transcription or DNA synthesis. Hybridization of labeled nucleic acids to microarrays is described (*e.g.*, in Schena, M. *et al.*(1995) *supra*; Wodicka, L. *et al.*(1997), *supra*; and DeSaizieu A. *et al.*(1998), *supra*). The detection and quantification of the hybridized molecule are tailored to the specific incorporated label. Radioactive labels can be detected, for example, as described in Schena, M. *et al.*(1995) *supra*) and fluorescent labels may be detected, for example, by the method of Shalon *et al.*(1996) *Genome Research* 6: 639-645).

The application of the sequences of the invention to DNA microarray technology, as described above, permits comparative analyses of different strains of *C. glutamicum* or other Corynebacteria. For example, studies of inter-strain variations based on individual transcript profiles and the identification of genes that are important for specific and/or desired strain properties such as pathogenicity, productivity and stress tolerance are facilitated by nucleic acid array methodologies. Also, comparisons of the profile of expression of genes of the invention during the course of a fermentation reaction are possible using nucleic acid array technology.

Example 13: Analysis of the Dynamics of Cellular Protein Populations (Proteomics)

The genes, compositions, and methods of the invention may be applied to study the interactions and dynamics of populations of proteins, termed 'proteomics'. Protein populations of interest include, but are not limited to, the total protein population of *C. glutamicum* (*e.g.*, in comparison with the protein populations of other organisms), those proteins which are active under specific environmental or metabolic conditions (*e.g.*, during fermentation, at high or low temperature, or at high or low pH), or those proteins which are active during specific phases of growth and development.

Protein populations can be analyzed by various well-known techniques, such as gel electrophoresis. Cellular proteins may be obtained, for example, by lysis or extraction, and may be separated from one another using a variety of electrophoretic techniques. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separates proteins largely on the basis of their molecular weight. Isoelectric focusing polyacrylamide gel electrophoresis (IEF-PAGE) separates proteins by their isoelectric point (which reflects not only the amino acid sequence but also posttranslational modifications of the protein). Another, more preferred method of protein analysis is the

consecutive combination of both IEF-PAGE and SDS-PAGE, known as 2-D-gel electrophoresis (described, for example, in Hermann *et al.*(1998) *Electrophoresis* 19: 3217-3221; Fountoulakis *et al.*(1998) *Electrophoresis* 19: 1193-1202; Langen *et al.*(1997) *Electrophoresis* 18: 1184-1192; Antelmann *et al.*(1997) *Electrophoresis* 18: 1451-1463). Other separation techniques may also be utilized for protein separation, such as capillary gel electrophoresis; such techniques are well known in the art.

Proteins separated by these methodologies can be visualized by standard techniques, such as by staining or labeling. Suitable stains are known in the art, and include Coomassie Brilliant Blue, silver stain, or fluorescent dyes such as Sypro Ruby (Molecular Probes). The inclusion of radioactively labeled amino acids or other protein precursors (*e.g.*, ^{35}S-methionine, ^{35}S-cysteine, ^{14}C-labelled amino acids, ^{15}N-amino acids, $^{15}NO_3$ or $^{15}NH_4^+$ or ^{13}C-labelled amino acids) in the medium of *C. glutamicum* permits the labeling of proteins from these cells prior to their separation. Similarly, fluorescent labels may be employed. These labeled proteins can be extracted, isolated and separated according to the previously described techniques.

Proteins visualized by these techniques can be further analyzed by measuring the amount of dye or label used. The amount of a given protein can be determined quantitatively using, for example, optical methods and can be compared to the amount of other proteins in the same gel or in other gels. Comparisons of proteins on gels can be made, for example, by optical comparison, by spectroscopy, by image scanning and analysis of gels, or through the use of photographic films and screens. Such techniques are well-known in the art.

To determine the identity of any given protein, direct sequencing or other standard techniques may be employed. For example, N- and/or C-terminal amino acid sequencing (such as Edman degradation) may be used, as may mass spectrometry (in particular MALDI or ESI techniques (see, *e.g.*, Langen *et al.*(1997) *Electrophoresis* 18: 1184-1192)). The protein sequences provided herein can be used for the identification of *C. glutamicum* proteins by these techniques.

The information obtained by these methods can be used to compare patterns of protein presence, activity, or modification between different samples from various biological conditions (*e.g.*, different organisms, time points of fermentation, media conditions, or different biotopes, among others). Data obtained from such experiments alone, or in combination with other techniques, can be used for various applications, such as to compare the behavior of various organisms in a given (*e.g.*, metabolic) situation, to increase the productivity of strains which produce fine chemicals or to increase the efficiency of the production of fine chemicals.

Equivalents

Those of ordinary skill in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

09609385 - 050200

TABLE 1: GENES IN THE APPLICATION

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
1	2	RXA01278	GR00369	2425	299	Protein Translation Elongation Factor G (EF-G)
3	4	RXA01913	GR00547	1856	2680	Protein translation Elongation Factor Ts (EF-Ts)
5	6	RXN01559	VV0171	7795	5954	PROTEIN-EXPORT MEMBRANE PROTEIN SECD
7	8	F RXA00935	GR00254	654	4	PROTEIN-EXPORT MEMBRANE PROTEIN SECD
9	10	F RXA01559	GR00434	1983	1741	PROTEIN-EXPORT MEMBRANE PROTEIN SECD
11	12	RXA01558	GR00434	1735	527	PROTEIN-EXPORT MEMBRANE PROTEIN SECF
13	14	RXA02429	GR00707	4823	7111	PREPROTEIN TRANSLOCASE SECA SUBUNIT
15	16	RXA02748	GR00764	2434	4074	SIGNAL RECOGNITION PARTICLE PROTEIN
17	18	RXA01355	GR00393	2877	3662	SIGNAL PEPTIDASE I (EC 3.4.21.89)
19	20	RXA00107	GR00014	17940	18176	GLUTAREDOXIN-LIKE PROTEIN NRDH
21	22	RXA01613	GR00449	7055	5841	GLUTATHIONE REDUCTASE (EC 1.6.4.2)
23	24	RXA00539	GR00139	1460	1936	GLUTATHIONE PEROXIDASE (EC 1.11.1.9)

Genes and enzymes involved in DNA uptake, repair and recombination

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
25	26	RXA01020	GR00291	998	1744	URACIL-DNA GLYCOSYLASE (EC 3.2.2.-)
27	28	RXN00484	VV0086	47365	46286	DEOXYRIBODIPYRIMIDINE PHOTOLYASE (EC 4.1.99.3)
29	30	F RXA00484	GR00119	21602	20568	DEOXYRIBODIPYRIMIDINE PHOTOLYASE (EC 4.1.99.3)
31	32	RXA02476	GR00715	10514	9636	A/G-SPECIFIC ADENINE GLYCOSYLASE (EC 3.2.2.-)
33	34	RXA00102	GR00014	11288	10521	FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE (EC 3.2.2.23)
35	36	RXN01670	VV0079	18911	18105	FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE (EC 3.2.2.23)
37	38	F RXA01670	GR00466	3	614	FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE (EC 3.2.2.23)
39	40	RXA02078	GR00628	8170	9027	FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE (EC 3.2.2.23)
41	42	RXA01596	GR00447	4370	6148	DNA REPAIR PROTEIN RECN
43	44	RXA01493	GR00423	7530	6220	DNA-DAMAGE-INDUCIBLE PROTEIN F
45	46	RXA02671	GR00753	11718	12296	DNA REPAIR PROTEIN RADA HOMOLOG
47	48	RXN02291	VV0127	18678	18025	ALKB PROTEIN (DNA repair – alkylated DNA)
49	50	F RXA02291	GR00662	1518	865	DNA repair gene specific for alkylated DNA
51	52	RXN01733	VV0221	70	1251	RECF PROTEIN
53	54	F RXA01733	GR00492	2	544	RECF PROTEIN
55	56	RXA01252	GR00365	643	1296	RECOMBINATION PROTEIN RECR
57	58	RXA01878	GR00537	1239	2117	DIMETHYLADENOSINE TRANSFERASE (EC 2.1.1.-)
59	60	RXA01556	GR00433	1	849	METHYLPHOSPHOTRIESTER-DNA ALKYLTRANSFERASE
61	62	RXA00053	GR00008	8162	8554	MUTATOR MUTT PROTEIN (7,8-DIHYDRO-8-OXOGUANINE-TRIPHOSPHATASE) (8-OXO-DGTPASE) (EC 3.6.1.-)
63	64	RXA00280	GR00043	4196	4696	MUTATOR MUTT PROTEIN (7,8-DIHYDRO-8-OXOGUANINE-TRIPHOSPHATASE) (8-OXO-DGTPASE) (EC 3.6.1.-)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
65	66	RXA00333	GR00057	16166	16699	MUTATOR MUTT PROTEIN (7,8-DIHYDRO-8-OXOGUANINE-TRIPHOSPHATASE) (8-OXO-DGTPASE) (EC 3.6.1.-)
67	68	RXA02110	GR00632	3641	4258	MUTATOR MUTT PROTEIN (7,8-DIHYDRO-8-OXOGUANINE-TRIPHOSPHATASE) (8-OXO-DGTPASE) (EC 3.6.1.-)
69	70	RXA02290	GR00662	693	295	DNA-3-METHYLADENINE GLYCOSIDASE I (EC 3.2.2.20)
71	72	RXA02557	GR00731	3766	3179	DNA-3-METHYLADENINE GLYCOSIDASE I (EC 3.2.2.20)
73	74	RXA02130	GR00638	1	87	DNA REPAIR HELICASE RAD25
75	76	RXA02742	GR00763	12384	10036	Hypothetical DNA Repair Helicase
77	78	RXA02445	GR00709	9362	11050	ATP-DEPENDENT DNA HELICASE RECG
79	80	RXA00927	GR00253	1606	518	HOLLIDAY JUNCTION DNA HELICASE RUVB
81	82	RXA00928	GR00253	2233	1616	HOLLIDAY JUNCTION DNA HELICASE RUVA
83	84	RXN00172	VV0187	7949	8560	RESOLVASE
85	86	F RXA00172	GR00027	455	6	RESOLVASE
87	88	RXA00184	GR00028	8239	9411	DNA repair exonuclease
89	90	RXA00019	GR00002	14399	16258	SINGLE-STRANDED-DNA-SPECIFIC EXONUCLEASE RECJ (EC 3.1.-.-)
91	92	RXA00929	GR00253	2938	2276	CROSSOVER JUNCTION ENDODEOXYRIBONUCLEASE RUVC (EC 3.1.22.4)
93	94	RXA02251	GR00654	18367	18666	EXCINUCLEASE ABC SUBUNIT C
95	96	RXA02252	GR00654	18632	20455	EXCINUCLEASE ABC SUBUNIT C
97	98	RXN02416	VV0116	10457	7629	EXCINUCLEASE ABC SUBUNIT A
99	100	F RXA02416	GR00705	3	2642	EXCINUCLEASE ABC SUBUNIT A
101	102	RXA02563	GR00732	1515	2246	Excinuclease ATPase subunit
103	104	RXA02731	GR00762	3263	5359	EXCINUCLEASE ABC SUBUNIT B
105	106	RXA00998	GR00283	2871	2410	COMA OPERON PROTEIN 2
107	108	RXN02386	VV0176	368	826	COME OPERON PROTEIN 1
109	110	F RXA02386	GR00693	1180	776	COME OPERON PROTEIN 1, DNA binding and uptake (competence)
111	112	RXN02388	VV0176	826	2487	COME OPERON PROTEIN 3
113	114	F RXA02385	GR00693	776	6	COME OPERON PROTEIN 3, DNA binding and uptake (competence)
115	116	F RXA02388	GR00694	1770	925	COME OPERON PROTEIN 3, DNA binding and uptake (competence)
117	118	RXA01975	GR00571	242	2137	PUTATIVE TYPE II RESTRICTION ENDONUCLEASE AND PUTATIVE TYPE I OR TYPE III RESTRICTION ENDONUCLEASE GENES, COMPLETE CDS
119	120	RXA01954	GR00562	3326	4165	TYPE III RESTRICTION-MODIFICATION SYSTEM ECOP15I ENZYME MOD (EC 2.1.1.72)
121	122	RXA02236	GR00654	4249	4566	integration host factor
123	124	RXN01795	VV0093	722	1318	MODIFICATION METHYLASE (EC 2.1.1.73)
125	126	RXN02267	VV0020	10928	10056	DNA (CYTOSINE-5)-METHYLTRANSFERASE (EC 2.1.1.37)
127	128	RXA02988	VV0093	231	836	MODIFICATION METHYLASE SCRFI-A (EC 2.1.1.73)
129	130	RXN00127	VV0124	9789	10253	COMPETENCE PROTEIN F
131	132	RXN02938	VV0054	23357	24097	MUTATOR MUTT PROTEIN (7,8-DIHYDRO-8-OXOGUANINE-TRIPHOSPHATASE) (8-OXO-DGTPASE)
133	134	RXN03102	VV0067	5253	4762	PUTATIVE COMPETENCE-DAMAGE PROTEIN
135	136	RXN03118	VV0093	1330	2139	PUTATIVE TYPE II RESTRICTION ENDONUCLEASE AND PUTATIVE TYPE I OR TYPE III RESTRICTION ENDONUCLEASE GENES, COMPLETE CDS
137	138	RXN02989	VV0073	118	1257	RECA PROTEIN
139	140	RXN03168	VV0327	1777	695	RIBONUCLEASE BN (EC 3.1.-.-)
141	142	RXN02431	VV0090	1	876	UMUC PROTEIN
143	144	RXN02985	VV0009	1182	850	EBSC PROTEIN

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
145	146	RXN02986	VV0009	801	664	EBSC PROTEIN
147	148	RXS00061	VV0044	4256	1590	DNA POLYMERASE I (EC 2.7.7.7)
149	150	RXS00212	VV0096	12413	10854	DNA LIGASE (EC 6.5.1.2)
151	152	RXS00213	VV0096	12894	12322	DNA LIGASE (EC 6.5.1.2)
153	154	RXS00724	VV0052	1217	3193	ATP-DEPENDENT DNA HELICASE RECG (EC 3.6.1.-)
155	156	RXS00823	VV0054	22014	22793	ENDONUCLEASE III (EC 4.2.99.18)
157	158	RXS00898	VV0140	4755	5543	EXODEOXYRIBONUCLEASE III (EC 3.1.11.2)
159	160	RXS01066	VV0099	21112	21837	DNA REPAIR PROTEIN RECO
161	162	RXS02145	VV0300	12248	13864	ENDONUCLEASE III (EC 4.2.99.18)
163	164	RXS02476	VV0008	49453	48575	A/G-SPECIFIC ADENINE GLYCOSYLASE (EC 3.2.2.-)
165	166	RXS02990	VV0073	1352	1948	REGULATORY PROTEIN RECX
167	168	RXS03098	VV0064	2100	2723	DNA alkylation repair enzyme
169	170	RXS03175	VV0331	1248	466	EXODEOXYRIBONUCLEASE III (EC 3.1.11.2)

Transposon, IS elements, Transposase, Integrase

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
171	172	RXN03069	VV0039	5816	4734	INTEGRASE
173	174	F RXA02890	GR10027	112	1194	INTEGRASE
175	176	RXA01601	GR00447	11128	12039	INTEGRASE/RECOMBINASE XERD
177	178	RXA01228	GR00355	1668	1883	TRANSPOSONS TN1721 AND TN4653 RESOLVASE
179	180	RXN03130	VV0123	14262	15569	DNA, TRANSPOSABLE ELEMENT IS31831
181	182	RXN01969	VV0155	139	504	DNA, TRANSPOSABLE ELEMENT IS31831
183	184	F RXA00263	GR00040	2243	936	DNA, TRANSPOSABLE ELEMENT IS31831
185	186	RXN01541	VV0015	56012	56788	PLASMID PASU1 TRANSPOSASE
187	188	F RXA01541	GR00428	3865	3095	PLASMID PASU1 TRANSPOSASE
189	190	RXA02590	GR00741	14837	13902	INSERTION ELEMENT IS1415 TRANSPOSASE (ISTA) AND HELPER PROTEIN (ISTB) GENES, COMPLETE CDS
191	192	RXA00016	GR00002	8857	7964	IS3 RELATED INSERTION ELEMENT
193	194	RXA00265	GR00040	2840	3289	TRANSPOSASE
195	196	RXA00938	GR00256	670	927	TRANSPOSASE
197	198	RXA01264	GR00367	12003	11788	TRANSPOSASE
199	200	RXA01265	GR00367	12616	12467	TRANSPOSASE
201	202	RXA01327	GR00386	753	896	TRANSPOSASE
203	204	RXA01328	GR00386	991	1365	TRANSPOSASE
205	206	RXA01329	GR00386	1407	1697	TRANSPOSASE
207	208	RXA01443	GR00418	13570	12740	TRANSPOSASE
209	210	RXA01444	GR00418	13928	13662	TRANSPOSASE
211	212	RXA01648	GR00457	829	461	TRANSPOSASE
213	214	RXA01649	GR00457	1260	841	TRANSPOSASE
215	216	RXA01650	GR00457	1437	1324	TRANSPOSASE
217	218	RXA01651	GR00457	1618	1484	TRANSPOSASE
219	220	RXN01680	VV0179	17470	17060	TRANSPOSASE

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
221	222	F RXA01680	GR00467	9590	9180	TRANSPOSASE
223	224	RXN01784	VV0084	13161	12580	TRANSPOSASE
225	226	F RXA01784	GR00505	3	551	TRANSPOSASE
227	228	RXA01862	GR00529	4961	6166	TRANSPOSASE
229	230	RXA01953	GR00562	928	548	TRANSPOSASE
231	232	RXA01998	GR00589	1345	2052	TRANSPOSASE
233	234	RXA02837	GR00829	179	6	TRANSPOSASE
235	236	RXA00005	GR00001	4724	6331	TRANSPOSASE
237	238	RXA00017	GR00002	9150	8857	TRANSPOSASE
239	240	RXA00057	GR00009	2491	2393	TRANSPOSASE
241	242	RXA00227	GR00032	27991	27194	TRANSPOSASE
243	244	RXA01819	GR00515	8287	7841	transposase
245	246	RXN03052	VV0024	5310	4555	INTEGRASE
247	248	RXN02915	VV0135	43798	44175	TRANSPOSASE
249	250	RXN02919	VV0084	14953	15486	TRANSPOSASE
251	252	RXN03033	VV0012	3942	5099	TRANSPOSASE
253	254	RXN03035	VV0013	667	1824	TRANSPOSASE
255	256	RXN03049	VV0020	29926	28985	TRANSPOSASE
257	258	RXN03070	VV0039	8897	8070	TRANSPOSASE
259	260	RXN03121	VV0101	645	4	TRANSPOSASE
261	262	RXN03161	VV0193	884	1267	TRANSPOSASE
263	264	RXN03165	VV0312	1562	1242	TRANSPOSASE
265	266	RXN00083	VV0048	3416	3117	TRANSPOSASE
267	268	RXN02004	VV0290	588	382	TRANSPOSASE
269	270	RXN02287	VV0127	69201	69752	TRANSPOSON TN2501 RESOLVASE
271	272	RXN02963	VV0102	6547	5240	DNA, TRANSPOSABLE ELEMENT IS31831

Aminoacyl-tRNA synthetases / tRNAs and tRNA metabolism

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
273	274	RXA02788	GR00777	2359	5022	ALANYL-TRNA SYNTHETASE (EC 6.1.1.7)
275	276	RXN00975	VV0149	7820	9469	ARGINYL-TRNA SYNTHETASE (EC 6.1.1.19)
277	278	F RXA00975	GR00275	780	4	POSSIBLE ARGINYL-TRNA SYNTHETASE (EC 6.1.1.19)
279	280	F RXA00976	GR00275	1423	824	POSSIBLE ARGINYL-TRNA SYNTHETASE (EC 6.1.1.19)
281	282	RXN01730	VV0137	1709	6	ASPARTYL-TRNA SYNTHETASE (EC 6.1.1.12)
283	284	F RXA01730	GR00490	298	1974	ASPARTYL-TRNA SYNTHETASE (EC 6.1.1.12)
285	286	RXA00314	GR00053	5406	4027	CYSTEINYL-TRNA SYNTHETASE (EC 6.1.1.16)
287	288	RXA02204	GR00646	8756	7497	CYSTEINYL-TRNA SYNTHETASE (EC 6.1.1.16)
289	290	RXA01124	GR00312	2	1510	GLUTAMYL-TRNA SYNTHETASE (EC 6.1.1.17)
291	292	RXN00458	VV0076	8169	8804	GLUTAMYL-TRNA SYNTHETASE (EC 6.1.1.17)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
293	294	F RXA00458	GR00115	232	5	GLUTAMYL-TRNA SYNTHETASE (EC 6.1.1.17)
295	296	RXA00069	GR00011	2782	1400	GLYCYL-TRNA SYNTHETASE (EC 6.1.1.14)
297	298	RXA01852	GR00525	4873	3587	HISTIDYL-TRNA SYNTHETASE (EC 6.1.1.21)
299	300	RXA02726	GR00760	4530	1597	ISOLEUCYL-TRNA SYNTHETASE (EC 6.1.1.5)
301	302	RXN00966	VV0262	543	4	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
303	304	F RXA00966	GR00271	533	6	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
305	306	RXN01061	VV0079	1	1038	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
307	308	F RXA01864	GR00531	474	4	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
309	310	F RXA01061	GR00296	10974	10567	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
311	312	RXA00968	GR00272	1007	6	LEUCYL-TRNA SYNTHETASE (EC 6.1.1.4)
313	314	RXA01522	GR00424	26014	27591	LYSYL-TRNA SYNTHETASE (EC 6.1.1.6)
315	316	RXA02015	GR00609	152	670	METHIONYL-TRNA SYNTHETASE (EC 6.1.1.10)
317	318	RXA01582	GR00440	1619	2707	PHENYLALANYL-TRNA SYNTHETASE ALPHA CHAIN (EC 6.1.1.20)
319	320	RXN01583	VV0122	19884	17542	PHENYLALANYL-TRNA SYNTHETASE BETA CHAIN (EC 6.1.1.20)
321	322	F RXA01583	GR00440	2914	4629	PHENYLALANYL-TRNA SYNTHETASE BETA CHAIN (EC 6.1.1.20)
323	324	F RXA01717	GR00487	1000	719	PHENYLALANYL-TRNA SYNTHETASE BETA CHAIN (EC 6.1.1.20)
325	326	RXN01938	VV0139	19106	20533	PROLYL-TRNA SYNTHETASE (EC 6.1.1.15)
327	328	F RXA01938	GR00556	94	1008	PROLYL-TRNA SYNTHETASE (EC 6.1.1.15)
329	330	RXA02692	GR00754	15485	16750	SERYL-TRNA SYNTHETASE (EC 6.1.1.11)
331	332	RXA02167	GR00640	13255	14514	TYROSYL-TRNA SYNTHETASE 1 (EC 6.1.1.1)
333	334	RXA02509	GR00721	2	1972	THREONYL-TRNA SYNTHETASE (EC 6.1.1.3)
335	336	RXN03169	VV0327	2326	1889	TRYPTOPHANYL-TRNA SYNTHETASE (EC 6.1.1.2)
337	338	F RXA02860	GR10006	2	439	TRYPTOPHANYL-TRNA SYNTHETASE (EC 6.1.1.2)
339	340	RXN03078	VV0045	484	5	TRYPTOPHANYL-TRNA SYNTHETASE (EC 6.1.1.2)
341	342	F RXA02866	GR10007	3992	4471	TRYPTOPHANYL-TRNA SYNTHETASE (EC 6.1.1.2)
343	344	RXN00985	VV0123	6747	9455	VALYL-TRNA SYNTHETASE (EC 6.1.1.9)
345	346	F RXA00985	GR00279	498	4	VALYL-TRNA SYNTHETASE (EC 6.1.1.9)
347	348	F RXA01347	GR00391	3036	5084	VALYL-TRNA SYNTHETASE (EC 6.1.1.9)
349	350	RXN00454	VV0076	3497	4789	QUEUINE TRNA-RIBOSYLTRANSFERASE (EC 2.4.2.29)
351	352	F RXA00454	GR00112	869	6	QUEUINE TRNA-RIBOSYLTRANSFERASE (EC 2.4.2.29)
353	354	RXN01490	VV0139	38695	37805	TRNA PSEUDOURIDINE SYNTHASE B (EC 4.2.1.70)
355	356	F RXA01490	GR00423	3442	4332	TRNA PSEUDOURIDINE 55 SYNTHASE
357	358	RXA01621	GR00452	473	1912	TRNA NUCLEOTIDYLTRANSFERASE (EC 2.7.7.25)
359	360	RXN01704	VV0191	1	1077	TRNA (URACIL-5-) -METHYLTRANSFERASE (EC 2.1.1.35)
361	362	F RXA01704	GR00480	3	818	TRNA (URACIL-5-) -METHYLTRANSFERASE (EC 2.1.1.35)
363	364	RXA02523	GR00725	1587	2405	TRNA (GUANINE-N1) -METHYLTRANSFERASE (EC 2.1.1.31)
365	366	RXA02243	GR00654	11114	12058	METHIONYL-TRNA FORMYLTRANSFERASE (EC 2.1.2.9)
367	368	RXA00217	GR00032	17389	16295	PROBABLE TRNA (5-METHYLAMINOMETHYL-2-THIOURIDYLATE) - METHYLTRANSFERASE (EC 2.1.1.61)
369	370	RXA01223	GR00354	4156	3545	PEPTIDYL-TRNA HYDROLASE (EC 3.1.1.29)
371	372	RXA01226	GR00354	7416	6973	PEPTIDYL-TRNA HYDROLASE (EC 3.1.1.29)
373	374	RXA00209	GR00032	9592	8102	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit A (EC 6.3.5.-)
375	376	RXA00210	GR00032	9897	9601	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit C (EC 6.3.5.-)
377	378	RXA02686	GR00754	11266	10130	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit A (EC 6.3.5.-)
379	380	RXA02625	GR00747	791	6	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit B (EC 6.3.5.-)
381	382	RXA01398	GR00408	7645	7010	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit B (EC 6.3.5.-)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
383	384	RXA02228	GR00653	1876	2778	TRNA DELTA(2)-ISOPENTENYLPYROPHOSPHATE TRANSFERASE (EC 2.5.1.8)
385	386	RXA02502	GR00720	15510	16901	GLUTAMYL-TRNA REDUCTASE (EC 1.2.1.-)
387	388	RXA02182	GR00641	17875	18648	GLUTAMINE CYCLOTRANSFERASE PRECURSOR (EC 2.3.2.5), Glutaminyl-tRNA cyclotransferase
389	390	RXN00211	VV0096	10126	10788	L-glutamyl-tRNA('Gln)-dependent amidotransferase subunit B (EC 6.3.5.-)
391	392	RXN00669	VV0005	38825	39706	PSEUDOURIDYLATE SYNTHASE I (EC 4.2.1.70)
393	394	RXN02651	VV0090	6842	7771	SFHB PROTEIN

Transcription

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
395	396	RXA01344	GR00390	2551	5	DNA-DIRECTED RNA POLYMERASE BETA CHAIN (EC 2.7.7.6)
397	398	RXA01387	GR00407	372	4	DNA-DIRECTED RNA POLYMERASE BETA' CHAIN (EC 2.7.7.6)
399	400	RXA01388	GR00407	590	459	DNA-DIRECTED RNA POLYMERASE BETA CHAIN (EC 2.7.7.6)
401	402	RXA01283	GR00369	7109	5817	DNA-DIRECTED RNA POLYMERASE BETA' CHAIN (EC 2.7.7.6)
403	404	RXA01433	GR00417	9606	9004	SIGMA FACTOR
405	406	RXA02456	GR00712	1127	510	RNA POLYMERASE SIGMA-H FACTOR
407	408	RXA00304	GR00051	696	4	RNA POLYMERASE SIGMA FACTOR
409	410	RXA00495	GR00123	1210	1773	PUTATIVE RNA POLYMERASE SIGMA FACTOR CY78.15
411	412	RXA00532	GR00137	3	587	PROBABLE RNA POLYMERASE SIGMA FACTOR CY49.08
413	414	RXA01530	GR00426	1724	1083	RNA POLYMERASE SIGMA FACTOR RPOD
415	416	RXA01531	GR00426	2565	1549	RNA POLYMERASE SIGMA FACTOR RPOD
417	418	RXA02065	GR00626	5348	5995	EXTRACYTOPLASMIC FUNCTION ALTERNATIVE SIGMA FACTOR
419	420	RXA00588	GR00156	13672	14193	TRANSCRIPTION ELONGATION FACTOR GREA
421	422	RXN01724	VV0037	2128	809	TRANSCRIPTION TERMINATION FACTOR RHO
423	424	F RXA01723	GR00488	6600	7436	TRANSCRIPTION TERMINATION FACTOR RHO
425	426	F RXA01724	GR00488	7429	7812	TRANSCRIPTION TERMINATION FACTOR RHO
427	428	RXN01725	VV0037	825	619	TRANSCRIPTION TERMINATION FACTOR RHO
429	430	F RXA01725	GR00488	7897	8004	TRANSCRIPTION TERMINATION FACTOR RHO
431	432	RXA01726	GR00488	8000	8572	TRANSCRIPTION TERMINATION FACTOR RHO
433	434	RXA00736	GR00199	1	1887	TRANSCRIPTION-REPAIR COUPLING FACTOR
435	436	RXN00737	VV0094	2673	1681	TRANSCRIPTION-REPAIR COUPLING FACTOR
437	438	F RXA00737	GR00200	1	480	TRANSCRIPTION-REPAIR COUPLING FACTOR
439	440	RXN01872	VV0248	2141	2968	TRANSCRIPTIONAL REGULATORY PROTEIN
441	442	F RXA01872	GR00535	768	4	TRANSCRIPTIONAL REGULATORY PROTEIN
443	444	RXA02413	GR00703	3029	2538	PAPX PROTEIN, transcriptional regulator
445	446	RXN01404	VV0278	3	1001	TRANSCRIPTION REGULATORY PROTEIN PEPR1
447	448	RXN02827	VV0350	428	6	TRANSCRIPTION-REPAIR COUPLING FACTOR
449	450	RXN02732	VV0145	3915	3475	Putative transcription factors
451	452	RXN01671	VV0079	17865	16717	RTCB PROTEIN
453	454	RXS00671	VV0005	37121	38134	DNA-DIRECTED RNA POLYMERASE ALPHA CHAIN (EC 2.7.7.6)
455	456	RXS02760	VV0025	31807	32760	TRANSCRIPTION ANTITERMINATION PROTEIN NUSG
457	458	RXS02830	VV0168	3	650	Helix-turn-helix domain-containing transcription regulator
459	460	RXS03207				RNA POLYMERASE SIGMA FACTOR

Translation

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
461	462	RXA02418	GR00705	5101	5667	Bacterial Protein Translation Initiation Factor 3 (IF-3)
463	464	RXN01496	VV0139	29945	32956	Protein Translation Initiation Factor 2 (IF-2)
465	466	F RXA00755	GR00203	1280	6	Protein Translation Initiation Factor 2 (IF-2)
467	468	F RXA01496	GR00423	10908	9181	Protein Translation Initiation Factor 2 (IF-2)
469	470	RXA00677	GR00178	1624	1839	Bacterial Protein Translation Initiation Factor 1 (IF-1)
471	472	RXN01284	VV0212	570	4	Bacterial Protein Translation Elongation Factor Tu (EF-TU)
473	474	F RXA01284	GR00370	510	4	Bacterial Protein Translation Elongation Factor Tu (EF-TU)
475	476	RXA00138	GR00022	1914	2474	Protein Translation Elongation Factor P (EF-P)
477	478	RXA00331	GR00057	15141	14785	Hypothetical Translational Inhibitor Protein
479	480	RXA02822	GR00803	1	570	Bacterial Peptide Chain Release Factor 1 (RF-1)
481	482	RXA00011	GR00002	2739	2383	Bacterial Peptide Chain Release Factor 2 (RF-2)
483	484	RXA00012	GR00002	3487	2612	Bacterial Peptide Chain Release Factor 2 (RF-2)
485	486	RXN01926	VV0284	1	741	PEPTIDE CHAIN RELEASE FACTOR 3
487	488	F RXA01926	GR00554	1	672	PEPTIDE CHAIN RELEASE FACTOR 3
489	490	RXN02002	VV0111	141	518	PEPTIDE CHAIN RELEASE FACTOR 3
491	492	F RXA02002	GR00592	383	6	PEPTIDE CHAIN RELEASE FACTOR 3
493	494	RXA00896	GR00244	2884	3522	POLYPEPTIDE DEFORMYLASE (EC 3.5.1.31)
495	496	RXA02242	GR00654	10585	11091	POLYPEPTIDE DEFORMYLASE (EC 3.5.1.31)
497	498	RXS02308	VV0127	13155	12727	TRANSLATION INITIATION INHIBITOR

Protein translocation, secretion, and folding

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
499	500	RXA01710	GR00484	850	443	PEPTIDE METHIONINE SULFOXIDE REDUCTASE
501	502	RXN02462	VV0124	11932	13749	PREPROTEIN TRANSLOCASE SECA SUBUNIT
503	504	F RXA00124	GR00020	737	6	PREPROTEIN TRANSLOCASE SECA SUBUNIT
505	506	F RXA02462	GR00712	7653	6739	PREPROTEIN TRANSLOCASE SECA SUBUNIT
507	508	RXA00125	GR00020	1467	703	PREPROTEIN TRANSLOCASE SECA SUBUNIT
509	510	RXA00687	GR00179	9121	10440	PREPROTEIN TRANSLOCASE SECY SUBUNIT
511	512	RXA02260	GR00654	30280	30510	PROTEIN-EXPORT MEMBRANE PROTEIN SECG HOMOLOG
513	514	RXN00046	VV0119	5363	6058	Signal recognition particle GTPase
515	516	F RXA00046	GR00007	5363	6058	Signal recognition particle GTPase
517	518	RXA00753	GR00202	23301	21880	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
519	520	RXN03038	VV0017	42941	43666	PS1 PROTEIN PRECURSOR
521	522	F RXA01179	GR00335	4639	5151	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
523	524	RXA01274	GR00367	27148	28242	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
525	526	RXA01449	GR00419	1046	6	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
527	528	RXA01798	GR00509	276	4	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
529	530	RXA01818	GR00515	6453	7439	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
531	532	RXA02607	GR00742	13971	14189	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
533	534	RXA02608	GR00742	14248	15942	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
535	536	RXN03054	VV0026	1906	3486	PS1 PROTEIN PRECURSOR
537	538	F RXA02886	GR10021	1907	2737	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
539	540	RXN03039	VV0018	2	631	PS1 PROTEIN PRECURSOR
541	542	F RXA02894	GR10036	1017	232	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
543	544	F RXA02904	GR10042	686	12	PS1 PROTEIN PRECURSOR (PS1, one of the two major secreted proteins of Corynebacterium glutamicum)
545	546	RXA02025	GR00614	862	212	PEPTIDE METHIONINE SULFOXIDE REDUCTASE
547	548	RXA01431	GR00417	7858	7538	THIOREDOXIN REDUCTASE (EC 1.6.4.5) / THIOREDOXIN
549	550	RXA01432	GR00417	8896	7946	THIOREDOXIN REDUCTASE (EC 1.6.4.5)
551	552	RXN00937	VV0079	42335	42706	THIOREDOXIN
553	554	F RXA00937	GR00256	1	123	THIOREDOXIN
555	556	RXA01199	GR00343	3813	4583	THIOREDOXIN
557	558	RXA00824	GR00221	4356	4913	THIOL:DISULFIDE INTERCHANGE PROTEIN TLPA
559	560	RXA01841	GR00522	115	477	THIOL:DISULFIDE INTERCHANGE PROTEIN TLPA
561	562	RXN01863	VV0206	1172	24	/O/C Thioredoxin-like oxidoreductases
563	564	F RXA01863	GR00530	830	24	/O/C Thioredoxin-like oxidoreductases
565	566	RXA02323	GR00668	1429	506	THIOREDOXIN REDUCTASE (EC 1.6.4.5)
567	568	RXA01072	GR00300	377	147	NRDH-REDOXIN
569	570	RXA02436	GR00709	1596	1036	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (EC 5.2.1.8)
571	572	RXN01837	VV0320	7103	7879	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (EC 5.2.1.8)
573	574	F RXA01837	GR00518	858	466	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE (EC 5.2.1.8)
575	576	RXS01277	VV0009	32155	34158	PROLYL ENDOPEPTIDASE (EC 3.4.21.26)
577	578	F RXA02047	GR00624	1	192	PROLYL ENDOPEPTIDASE (EC 3.4.21.26)
579	580	RXA02174	GR00641	9290	8937	PROBABLE FK506-BINDING PROTEIN (PEPTIDYL-PROLYL CIS-TRANS ISOMERASE) (PPIASE) (EC 5.2.1.8)
581	582	RXA00568	GR00152	2928	1582	TRIGGER FACTOR
583	584	RXN03040	VV0018	761	1069	PS1 PROTEIN PRECURSOR
585	586	RXN03051	VV0022	2832	3566	PS1 PROTEIN PRECURSOR
587	588	RXN02949	VV0025	31243	31575	PREPROTEIN TRANSLOCASE SECE SUBUNIT
589	590	RXN00833	VV0180	8039	8533	THIOL PEROXIDASE (EC 1.11.1.-)
591	592	RXN01676	VV0179	12059	11304	THIOL:DISULFIDE INTERCHANGE PROTEIN DSBD
593	594	RXN00380	VV0223	836	216	THIOL:DISULFIDE INTERCHANGE PROTEIN TLPA

Nucleic Acid SEQ ID NO	Amino Acid SEQ ID NO	Identification Code	Contig.	NT Start	NT Stop	Function
595	596	RXN02325	VV0047	5527	6393	THIOREDOXIN
597	598	RXN00493	VV0086	14389	16002	60 KD CHAPERONIN
599	600	RXN02543	VV0057	22031	20178	DNAK PROTEIN
601	602	RXN01345	VV0123	4883	3432	Molecular chaperones (HSP70/DnaK family)
603	604	RXN02736	VV0074	13600	14556	PUTATIVE OXPPCYCLE PROTEIN OPCA
605	606	RXN02280	VV0152	1849	26	TRAP1
607	608	RXS00170	VV0031	4882	3029	PS1 PROTEIN PRECURSOR
609	610	RXS02641	VV0098	49070	51145	PS1 PROTEIN PRECURSOR
611	612	RXS02650	VV0090	6261	6839	LIPOPROTEIN SIGNAL PEPTIDASE (EC 3.4.23.36)
613	614	RXS00076	VV0154	2752	4122	NADPH:FERREODOXIN OXIDOREDUCTASE PRECURSOR (EC 1.18.1.2)
615	616	RXS01438	VV0089	25340	23976	NADPH:FERREODOXIN OXIDOREDUCTASE PRECURSOR (EC 1.18.1.2)

TABLE 2: GENES IDENTIFIED FROM GENBANK

GenBank™ Accession No.	Gene Name	Gene Function	Reference
A09073	ppg	Phosphoenol pyruvate carboxylase	Bachmann, B. et al. "DNA fragment coding for phosphoenolpyruvat corboxylase, recombinant DNA carrying said fragment, strains carrying the recombinant DNA and method for producing L-aminino acids using said strains," Patent: EP 0358940-A 3 03/21/90
A45579, A45581, [illegible]5583, [illegible]6585 A45587		Threonine dehydratase	Moeckel, B. et al. "Production of L-isoleucine by means of recombinant micro-organisms with deregulated threonine dehydratase," Patent: WO 9519442-A 5 07/20/95
AB003132	murC; ftsQ; ftsZ		Kobayashi, M. et al. "Cloning, sequencing, and characterization of the ftsZ gene from coryneform bacteria," *Biochem. Biophys. Res. Commun.*, 236(2):383-388 (1997)
AB015023	murC; ftsQ		Wachi, M. et al. "A murC gene from Coryneform bacteria," *Appl. Microbiol. Biotechnol.*, 51(2):223-228 (1999)
AB018530	dtsR		Kimura, E. et al. "Molecular cloning of a novel gene, dtsR, which rescues the detergent sensitivity of a mutant derived from *Brevibacterium lactofermentum*," *Biosci. Biotechnol. Biochem.*, 60(10):1565-1570 (1996)
AB018531	dtsR1; dtsR2		
AB020624	murI	D-glutamate racemase	
AB023377	tkt	transketolase	
AB024708	gltB; gltD	Glutamine 2-oxoglutarate aminotransferase large and small subunits	
AB025424	acn	aconitase	
[illegible]027714	rep	Replication protein	
[illegible]027715	rep; aad	Replication protein; aminoglycoside adenyltransferase	
AF005242	argC	N-acetylglutamate-5-semialdehyde dehydrogenase	
AF005635	glnA	Glutamine synthetase	
AF030405	hisF	cyclase	
AF030520	argG	Argininosuccinate synthetase	
AF031518	argF	Ornithine carbamolytransferase	
AF036932	aroD	3-dehydroquinate dehydratase	

GenBank™ Accession No.	Gene Name	Gene Function	Reference
AF038548	pyc	Pyruvate carboxylase	
AF038651	dciAE; apt; rel	Dipeptide-binding protein; adenine phosphoribosyltransferase; GTP pyrophosphokinase	Wehmeier, L. et al. "The role of the Corynebacterium glutamicum rel gene in (p)ppGpp metabolism," *Microbiology,* 144:1853-1862 (1998)
AF041436	argR	Arginine repressor	
AF045998	impA	Inositol monophosphate phosphatase	
[illegible]048764	argH	Argininosuccinate lyase	
[illegible]049897	argC; argJ; argB; argD; argF; argR; argG; argH	N-acetylglutamylphosphate reductase; ornithine acetyltransferase; N-acetylglutamate kinase; acetylornithine transminase; ornithine carbamoyltransferase; arginine repressor; argininosuccinate synthase; argininosuccinate lyase	
AF050109	inhA	Enoyl-acyl carrier protein reductase	
AF050166	hisG	ATP phosphoribosyltransferase	
AF051846	hisA	Phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazolecarboxamide isomerase	
AF052652	metA	Homoserine O-acetyltransferase	Park, S. et al. "Isolation and analysis of metA, a methionine biosynthetic gene encoding homoserine acetyltransferase in Corynebacterium glutamicum," *Mol. Cells.,* 8(3):286-294 (1998)
AF053071	aroB	Dehydroquinate synthetase	
[illegible]060558	hisH	Glutamine amidotransferase	
[illegible]086704	hisE	Phosphoribosyl-ATP-pyrophosphohydrolase	
AF114233	aroA	5-enolpyruvylshikimate 3-phosphate synthase	
AF116184	panD	L-aspartate-alpha-decarboxylase precursor	Dusch, N. et al. "Expression of the Corynebacterium glutamicum panD gene encoding L-aspartate-alpha-decarboxylase leads to pantothenate overproduction in Escherichia coli," *Appl. Environ. Microbiol.,* 65(4)1530-1539 (1999)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
AF124518	aroD; aroE	3-dehydroquinase; shikimate dehydrogenase	
AF124600	aroC; aroK; aroB; pepQ	Chorismate synthase; shikimate kinase; 3-dehydroquinate synthase; putative cytoplasmic peptidase	
AF145897	inhA		
[redacted]145898	inhA		
[redacted]001436	ectP	Transport of ectoine, glycine betaine, proline	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes: Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP," *J. Bacteriol.*, 180(22):6005-6012 (1998)
AJ004934	dapD	Tetrahydrodipicolinate succinylase (incomplete[i])	Wehrmann, A. et al. "Different modes of diaminopimelate synthesis and their role in cell wall integrity: A study with Corynebacterium glutamicum," *J. Bacteriol.*, 180(12):3159-3165 (1998)
AJ007732	ppc; secG; amt; ocd; soxA	Phosphoenolpyruvate-carboxylase; ?; high affinity ammonium uptake protein; putative ornithine-cyclodecarboxylase; sarcosine oxidase	
AJ010319	ftsY, glnB, glnD; srp; amtP	Involved in cell division; PII protein; uridylyltransferase (uridylyl-removing enzmye); signal recognition particle; low affinity ammonium uptake protein	Jakoby, M. et al. "Nitrogen regulation in Corynebacterium glutamicum; Isolation of genes involved in biochemical characterization of corresponding proteins," *FEMS Microbiol.*, 173(2):303-310 (1999)
AJ132968	cat	Chloramphenicol aceteyl transferase	
[redacted]24946	mqo	L-malate: quinone oxidoreductase	Molenaar, D. et al. "Biochemical and genetic characterization of the membrane-associated malate dehydrogenase (acceptor) from Corynebacterium glutamicum," *Eur. J. Biochem.*, 254(2):395-403 (1998)
AJ238250	ndh	NADH dehydrogenase	
AJ238703	porA	Porin	Lichtinger, T. et al. "Biochemical and biophysical characterization of the cell wall porin of Corynebacterium glutamicum: The channel is formed by a low molecular mass polypeptide," *Biochemistry*, 37(43):15024-15032 (1998)
D17429		Transposable element IS31831	Vertes, A.A. et al. "Isolation and characterization of IS31831, a transposable element from Corynebacterium glutamicum," *Mol. Microbiol.*, 11(4):739-746 (1994)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
D84102	odhA	2-oxoglutarate dehydrogenase	Usuda, Y. et al. "Molecular cloning of the Corynebacterium glutamicum (Brevibacterium lactofermentum AJ12036) odhA gene encoding a novel type of 2-oxoglutarate dehydrogenase," *Microbiology*, 142:3347-3354 (1996)
E01358	hdh; hk	Homoserine dehydrogenase; homoserine kinase	Katsumata, R. et al. "Production of L-thereonine and L-isoleucine," Patent: JP 1987232392-A 1 10/12/87
E01359		Upstream of the start codon of homoserine kinase gene	Katsumata, R. et al. "Production of L-thereonine and L-isoleucine," Patent: JP 1987232392-A 2 10/12/87
[redacted]1375		Tryptophan operon	
E01376	trpL; trpE	Leader peptide; anthranilate synthase	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87
E01377		Promoter and operator regions of tryptophan operon	Matsui, K. et al. "Tryptophan operon, peptide and protein coded thereby, utilization of tryptophan operon gene expression and production of tryptophan," Patent: JP 1987244382-A 1 10/24/87
E03937		Biotin-synthase	Hatakeyama, K. et al. "DNA fragment containing gene capable of coding biotin synthetase and its utilization," Patent: JP 1992278088-A 1 10/02/92
E04040		Diamino pelargonic acid aminotransferase	Kohama, K. et al. "Gene coding diaminopelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04041		Desthiobiotinsynthetase	Kohama, K. et al. "Gene coding diaminopelargonic acid aminotransferase and desthiobiotin synthetase and its utilization," Patent: JP 1992330284-A 1 11/18/92
E04307		Flavum aspartase	Kurusu, Y. et al. "Gene DNA coding aspartase and utilization thereof," Patent: JP 1993030977-A 1 02/09/93
[redacted]4376		Isocitric acid lyase	Katsumata, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993056782-A 3 03/09/93
E04377		Isocitric acid lyase N-terminal fragment	Katsumata, R. et al. "Gene manifestation controlling DNA," Patent: JP 1993056782-A 3 03/09/93
E04484		Prephenate dehydratase	Sotouchi, N. et al. "Production of L-phenylalanine by fermentation," Patent: JP 1993076352-A 2 03/30/93
E05108		Aspartokinase	Fugono, N. et al. "Gene DNA coding Aspartokinase and its use," Patent: JP 1993184366-A 1 07/27/93
E05112		Dihydro-dipichorinate synthetase	Hatakeyama, K. et al. "Gene DNA coding dihydrodipicolinic acid synthetase and its use," Patent: JP 1993184371-A 1 07/27/93

GenBank™ Accession No.	Gene Name	Gene Function	Reference
E05776		Diaminopimelic acid dehydrogenase	Kobayashi, M. et al. "Gene DNA coding Diaminopimelic acid dehydrogenase and its use," Patent: JP 1993284970-A 1 11/02/93
E05779		Threonine synthase	Kohama, K. et al. "Gene DNA coding threonine synthase and its use," Patent: JP 1993284972-A 1 11/02/93
E06110		Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent: JP 1993344881-A 1 12/27/93
[redacted]6111		Mutated Prephenate dehydratase	Kikuchi, T. et al. "Production of L-phenylalanine by fermentation method," Patent: JP 1993344881-A 1 12/27/93
E06146		Acetohydroxy acid synthetase	Inui, M. et al. "Gene capable of coding Acetohydroxy acid synthetase and its use," Patent: JP 1993344893-A 1 12/27/93
E06825		Aspartokinase	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94
E06826		Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94
E06827		Mutated aspartokinase alpha subunit	Sugimoto, M. et al. "Mutant aspartokinase gene," patent: JP 1994062866-A 1 03/08/94
E07701	secY		Honno, N. et al. "Gene DNA participating in integration of membraneous protein to membrane," Patent: JP 1994169780-A 1 06/21/94
E08177		Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent: JP 1994261766-A 1 09/20/94
E08178, E08179, [redacted]180, [redacted]8181, E08182		Feedback inhibition-released Aspartokinase	Sato, Y. et al. "Genetic DNA capable of coding Aspartokinase released from feedback inhibition and its utilization," Patent: JP 1994261766-A 1 09/20/94
E08232		Acetohydroxy-acid isomeroreductase	Inui, M. et al. "Gene DNA coding acetohydroxy acid isomeroreductase," Patent: JP 1994277067-A 1 10/04/94
E08234	secE		Asai, Y. et al. "Gene DNA coding for translocation machinery of protein," Patent: JP 1994277073-A 1 10/04/94
E08643		FT aminotransferase and desthiobiotin synthetase promoter region	Hatakeyama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031476-A 1 02/03/95
E08646		Biotin synthetase	Hatakeyama, K. et al. "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031476-A 1 02/03/95

GenBank™ Accession No.	Gene Name	Gene Function	Reference
E08649		Aspartase	Kohama, K. et al "DNA fragment having promoter function in coryneform bacterium," Patent: JP 1995031478-A 1 02/03/95
E08900		Dihydrodipicolinate reductase	Madori, M. et al. "DNA fragment containing gene coding Dihydrodipicolinate acid reductase and utilization thereof," Patent: JP 1995075578-A 1 03/20/95
E08901		Diaminopimelic acid decarboxylase	Madori, M. et al. "DNA fragment containing gene coding Diaminopimelic acid decarboxylase and utilization thereof," Patent: JP 1995075579-A 1 03/20/95
[redacted]2594		Serine hydroxymethyltransferase	Hatakeyama, K. et al. "Production of L-trypophan," Patent: JP 1997028391-A 1 02/04/97
E12760, E12759, E12758		transposase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12764		Arginyl-tRNA synthetase; diaminopimelic acid decarboxylase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12767		Dihydrodipicolinic acid synthetase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12770		aspartokinase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E12773		Dihydrodipicolinic acid reductase	Moriya, M. et al. "Amplification of gene using artificial transposon," Patent: JP 1997070291-A 03/18/97
E13655		Glucose-6-phosphate dehydrogenase	Hatakeyama, K. et al. "Glucose-6-phosphate dehydrogenase and DNA capable of coding the same," Patent: JP 1997224661-A 1 09/02/97
L01508	IlvA	Threonine dehydratase	Moeckel, B. et al. "Functional and structural analysis of the threonine dehydratase of Corynebacterium glutamicum," *J. Bacteriol.*, 174:8065-8072 (1992)
L07603	EC 4.2.1.15	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase	Chen, C. et al. "The cloning and nucleotide sequence of Corynebacterium glutamicum 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase gene," *FEMS Microbiol. Lett.*, 107:223-230 (1993)
L09232	IlvB; ilvN; ilvC	Acetohydroxy acid synthase large subunit; Acetohydroxy acid synthase small subunit; Acetohydroxy acid isomeroreductase	Keilhauer, C. et al. "Isoleucine synthesis in Corynebacterium glutamicum: molecular analysis of the ilvB-ilvN-ilvC operon," *J. Bacteriol.*, 175(17):5595-5603 (1993)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
L18874	PtsM	Phosphoenolpyruvate sugar phosphotransferase	Fouet, A et al. "Bacillus subtilis sucrose-specific enzyme II of the phosphotransferase system: expression in Escherichia coli and homology to enzymes II from enteric bacteria," *PNAS USA*, 84(24):8773-8777 (1987); Lee, J.K. et al. "Nucleotide sequence of the gene encoding the Corynebacterium glutamicum mannose enzyme II and analyses of the deduced protein sequence," *FEMS Microbiol. Lett.*, 119(1-2):137-145 (1994)
[redacted]7123	aceB	Malate synthase	Lee, H-S. et al. "Molecular characterization of aceB, a gene encoding malate synthase in Corynebacterium glutamicum," *J. Microbiol. Biotechnol.*, 4(4):256-263 (1994)
L27126		Pyruvate kinase	Jetten, M. S. et al. "Structural and functional analysis of pyruvate kinase from Corynebacterium glutamicum," *Appl. Environ. Microbiol.*, 60(7):2501-2507 (1994)
L28760	aceA	Isocitrate lyase	
L35906	dtxr	Diphtheria toxin repressor	Oguiza, J.A. et al. "Molecular cloning, DNA sequence analysis, and characterization of the Corynebacterium diphtheriae dtxR from Brevibacterium lactofermentum," *J. Bacteriol.*, 177(2):465-467 (1995)
M13774		Prephenate dehydratase	Follettie, M.T. et al. "Molecular cloning and nucleotide sequence of the Corynebacterium glutamicum pheA gene," *J. Bacteriol.*, 167:695-702 (1986)
M16175	5S rRNA		Park, Y-H. et al. "Phylogenetic analysis of the coryneform bacteria by 56 rRNA sequences," *J. Bacteriol.*, 169:1801-1806 (1987)
M16663	trpE	Anthranilate synthase, 5' end	Sano, K. et al. "Structure and function of the trp operon control regions of Brevibacterium lactofermentum, a glutamic-acid-producing bacterium," *Gene*, 52:191-200 (1987)
[redacted]6664	trpA	Tryptophan synthase, 3'end	Sano, K. et al. "Structure and function of the trp operon control regions of Brevibacterium lactofermentum, a glutamic-acid-producing bacterium," *Gene*, 52:191-200 (1987)
M25819		Phosphoenolpyruvate carboxylase	O'Regan, M. et al. "Cloning and nucleotide sequence of the Phosphoenolpyruvate carboxylase-coding gene of Corynebacterium glutamicum ATCC13032," *Gene*, 77(2):237-251 (1989)
M85106		23S rRNA gene insertion sequence	Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," *J. Gen. Microbiol.*, 138:1167-1175 (1992)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
M85107, M85108		23S rRNA gene insertion sequence	Roller, C. et al. "Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes," *J. Gen. Microbiol.*, 138:1167-1175 (1992)
M89931	aecD; brnQ; yhbw	Beta C-S lyase; branched-chain amino acid uptake carrier; hypothetical protein yhbw	Rossol, I. et al. "The Corynebacterium glutamicum aecD gene encodes a C-S lyase with alpha, beta-elimination activity that degrades aminoethylcysteine," *J. Bacteriol.*, 174(9):2968-2977 (1992); Tauch, A. et al. "Isoleucine uptake in Corynebacterium glutamicum ATCC 13032 is directed by the brnQ gene product," *Arch. Microbiol.*, 169(4):303-312 (1998)
S59299	trp	Leader gene (promoter)	Herry, D.M. et al. "Cloning of the trp gene cluster from a tryptophan-hyperproducing strain of Corynebacterium glutamicum: identification of a mutation in the trp leader sequence," *Appl. Environ. Microbiol.*, 59(3):791-799 (1993)
U11545	trpD	Anthranilate phosphoribosyltransferase	O'Gara, J.P. and Dunican, L.K. (1994) Complete nucleotide sequence of the Corynebacterium glutamicum ATCC 21850 tpD gene." Thesis, Microbiology Department, University College Galway, Ireland.
U13922	cglIM; cglIR; clgIIR	Putative type II 5-cytosoine methyltransferase; putative type II restriction endonuclease; putative type I or type III restriction endonuclease	Schafer, A. et al. "Cloning and characterization of a DNA region encoding a stress-sensitive restriction system from Corynebacterium glutamicum ATCC 13032 and analysis of its role in intergeneric conjugation with Escherichia coli," *J. Bacteriol.*, 176(23):7309-7319 (1994); Schafer, A. et al. "The Corynebacterium glutamicum cglIM gene encoding a 5-cytosine in an McrBC-deficient Escherichia coli strain," *Gene*, 203(2):95-101 (1997)
U14965	recA		
U31224	ppx		Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)
U31225	proC	L-proline: NADP+ 5-oxidoreductase	Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)
U31230	obg; proB; unkdh	?;gamma glutamyl kinase;similar to D-isomer specific 2-hydroxyacid dehydrogenases	Ankri, S. et al. "Mutations in the Corynebacterium glutamicumproline biosynthetic pathway: A natural bypass of the proA step," *J. Bacteriol.*, 178(15):4412-4419 (1996)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
U31281	bioB	Biotin synthase	Serebriiskii, I.G., "Two new members of the bio B superfamily: Cloning, sequencing and expression of bio B genes of Methylobacillus flagellatum and Corynebacterium glutamicum," *Gene,* 175:15-22 (1996)
U35023	thtR; accBC	Thiosulfate sulfurtransferase; acyl CoA carboxylase	Jager, W. et al. "A Corynebacterium glutamicum gene encoding a two-domain protein similar to biotin carboxylases and biotin-carboxyl-carrier proteins," *Arch. Microbiol.,* 166(2);76-82 (1996)
[redacted]3535	cmr	Multidrug resistance protein	Jager, W. et al. "A Corynebacterium glutamicum gene conferring multidrug resistance in the heterologous host Escherichia coli," *J. Bacteriol.,* 179(7):2449-2451 (1997)
U43536	clpB	Heat shock ATP-binding protein	
U53587	aphA-3	3'5''-aminoglycoside phosphotransferase	
U89648		Corynebacterium glutamicum unidentified sequence involved in histidine biosynthesis, partial sequence	
X04960	trpA; trpB; trpC; trpD; trpE; trpG; trpL	Tryptophan operon	Matsui, K. et al. "Complete nucleotide and deduced amino acid sequences of the Brevibacterium lactofermentum tryptophan operon," *Nucleic Acids Res.,* 14(24):10113-10114 (1986)
X07563	lys A	DAP decarboxylase (meso-diaminopimelate decarboxylase, EC 4.1.1.20)	Yeh, P. et al. "Nucleic sequence of the lysA gene of Corynebacterium glutamicum and possible mechanisms for modulation of its expression," *Mol. Gen. Genet.,* 212(1):112-119 (1988)
X14234	EC 4.1.1.31	Phosphoenolpyruvate carboxylase	Eikmanns, B.J. et al. "The Phosphoenolpyruvate carboxylase gene of Corynebacterium glutamicum: Molecular cloning, nucleotide sequence, and expression," *Mol. Gen. Genet.,* 218(2):330-339 (1989); Lepiniec, L. et al. "Sorghum Phosphoenolpyruvate carboxylase gene family: structure, function and molecular evolution," *Plant. Mol. Biol.,* 21 (3):487-502 (1993)
X17313	fda	Fructose-bisphosphate aldolase	Von der Osten, C.H. et al. "Molecular cloning, nucleotide sequence and fine-structural analysis of the Corynebacterium glutamicum fda gene: structural comparison of C. glutamicum fructose-1, 6-biphosphate aldolase to class I and class II aldolases," *Mol. Microbiol.,*
X53993	dapA	L-2, 3-dihydrodipicolinate synthetase (EC 4.2.1.52)	Bonnassie, S. et al. "Nucleic sequence of the dapA gene from Corynebacterium glutamicum," *Nucleic Acids Res.,* 18(21):6421 (1990)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X54223		AttB-related site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium glutamicum , and the attP site of lambdacorynephage," *FEMS. Microbiol, Lett.,* 66:299-302 (1990)
X54740	argS; lysA	Arginyl-tRNA synthetase; Diaminopimelate decarboxylase	Marcel, T. et al. "Nucleotide sequence and organization of the upstream region of the Corynebacterium glutamicum lysA gene," *Mol. Microbiol.,* 4(11):1819-1830 (1990)
[redacted]5994	trpL; trpE	Putative leader peptide; anthranilate synthase component 1	Heery, D.M. et al. "Nucleotide sequence of the Corynebacterium glutamicum trpE gene," *Nucleic Acids Res.,* 18(23):7138 (1990)
X56037	thrC	Threonine synthase	Han, K.S. et al. "The molecular structure of the Corynebacterium glutamicum threonine synthase gene," *Mol. Microbiol.,* 4(10):1693-1702 (1990)
X56075	attB-related site	Attachment site	Cianciotto, N. et al. "DNA sequence homology between att B-related sites of Corynebacterium diphtheriae, Corynebacterium ulcerans, Corynebacterium glutamicum , and the attP site of lambdacorynephage," *FEMS. Microbiol, Lett.,* 66:299-302 (1990)
X57226	lysC-alpha; lysC-beta; asd	Aspartokinase-alpha subunit; Aspartokinase-beta subunit; aspartate beta semialdehyde dehydrogenase	Kalinowski, J. et al. "Genetic and biochemical analysis of the Aspartokinase from Corynebacterium glutamicum," *Mol. Microbiol.,* 5(5):1197-1204 (1991); Kalinowski, J. et al. "Aspartokinase genes lysC alpha and lysC beta overlap and are adjacent to the aspertate beta-semialdehyde dehydrogenase gene asd in Corynebacterium glutamicum," *Mol. Gen. Genet.,* 224(3):317-324 (1990)
X59403	gap;pgk; tpi	Glyceraldehyde-3-phosphate; phosphoglycerate kinase; triosephosphate isomerase	Eikmanns, B.J. "Identification, sequence analysis, and expression of a Corynebacterium glutamicum gene cluster encoding the three glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and triosephosphate isomeras," *J. Bacteriol.,* 174(19):6076-6086 (1992)
X59404	gdh	Glutamate dehydrogenase	Bormann, E.R. et al. "Molecular analysis of the Corynebacterium glutamicum gdh gene encoding glutamate dehydrogenase," *Mol. Microbiol.,* 6(3):317-326 (1992)
X60312	lysI	L-lysine permease	Seep-Feldhaus, A.H. et al. "Molecular analysis of the Corynebacterium glutamicum lysI gene involved in lysine uptake," *Mol. Microbiol.,* 5(12):2995-3005 (1991)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X66078	cop1	Ps1 protein	Joliff, G. et al. "Cloning and nucleotide sequence of the csp1 gene encoding PS1, one of the two major secreted proteins of Corynebacterium glutamicum: The deduced N-terminal region of PS1 is similar to the Mycobacterium antigen 85 complex," *Mol. Microbiol.*, 6(16):2349-2362 (1992)
X66112	glt	Citrate synthase	Eikmanns, B.J. et al. "Cloning sequence, expression and transcriptional analysis of the Corynebacterium glutamicum gltA gene encoding citrate synthase," *Microbiol.*, 140:1817-1828 (1994)
[redacted]7737	dapB	Dihydrodipicolinate reductase	
X69103	csp2	Surface layer protein PS2	Peyret, J.L. et al. "Characterization of the cspB gene encoding PS2, an ordered surface-layer protein in Corynebacterium glutamicum," *Mol. Microbiol.*, 9(1):97-109 (1993)
X69104		IS3 related insertion element	Bonamy, C. et al. "Identification of IS1206, a Corynebacterium glutamicum IS3-related insertion sequence and phylogenetic analysis," *Mol. Microbiol.*, 14(3):571-581 (1994)
X70959	leuA	Isopropylmalate synthase	Patek, M. et al. "Leucine synthesis in Corynebacterium glutamicum: enzyme activities, structure of leuA, and effect of leuA inactivation on lysine synthesis," *Appl. Environ. Microbiol.*, 60(1):133-140 (1994)
X71489	icd	Isocitrate dehydrogenase (NADP+)	Eikmanns, B.J. et al. "Cloning sequence analysis, expression, and inactivation of the Corynebacterium glutamicum icd gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme," *J. Bacteriol.*, 177(3):774-782 (1995)
X72855	GDHA	Glutamate dehydrogenase (NADP+)	
X75083, [redacted]0584	mtrA	5-methyltryptophan resistance	Heery, D.M. et al. "A sequence from a tryptophan-hyperproducing strain of Corynebacterium glutamicum encoding resistance to 5-methyltryptophan," *Biochem. Biophys. Res. Commun.*, 201(3):1255-1262 (1994)
X75085	recA		Fitzpatrick, R. et al. "Construction and characterization of recA mutant strains of Corynebacterium glutamicum and Brevibacterium lactofermentum," *Appl. Microbiol. Biotechnol.*, 42(4):575-580 (1994)
X75504	aceA; thiX	Partial Isocitrate lyase; ?	Reinscheid, D.J. et al. "Characterization of the isocitrate lyase gene from Corynebacterium glutamicum and biochemical analysis of the enzyme," *J. Bacteriol.*, 176(12):3474-3483 (1994)
X76875		ATPase beta-subunit	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes," *Antonie Van Leeuwenhoek*, 64:285-305 (1993)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X77034	tuf	Elongation factor Tu	Ludwig, W. et al. "Phylogenetic relationships of bacteria based on comparative sequence analysis of elongation factor Tu and ATP-synthase beta-subunit genes," *Antonie Van Leeuwenhoek*, 64:285-305 (1993)
X77384	recA		Billman-Jacobe, H. "Nucleotide sequence of a recA gene from Corynebacterium glutamicum," *DNA Seq.*, 4(6):403-404 (1994)
X78491	aceB	Malate synthase	Reinscheid, D.J. et al. "Malate synthase from Corynebacterium glutamicum pta-ack operon encoding phosphotransacetylase: sequence analysis," *Microbiology*, 140:3099-3108 (1994)
X80629	16S rDNA	16S ribosomal RNA	Rainey, F.A. et al. "Phylogenetic analysis of the genera Rhodococcus and Norcardia and evidence for the evolutionary origin of the genus Norcardia from within the radiation of Rhodococcus species," *Microbiol.*, 141:523-528 (1995)
X81191	gluA; gluB; gluC; gluD	Glutamate uptake system	Kronemeyer, W. et al. "Structure of the gluABCD cluster encoding the glutamate uptake system of Corynebacterium glutamicum," *J. Bacteriol.*, 177(5):1152-1158 (1995)
X81379	dapE	Succinyldiaminopimelate desuccinylase	Wehrmann, A. et al. "Analysis of different DNA fragments of Corynebacterium glutamicum complementing dapE of Escherichia coli," *Microbiology*, 40:3349-56 (1994)
X82061	16S rDNA	16S ribosomal RNA	Ruimy, R. et al. "Phylogeny of the genus Corynebacterium deduced from analyses of small-subunit ribosomal DNA sequences," *Int. J. Syst. Bacteriol.*, 45(4):740-746 (1995)
X82928	asd; lysC	Aspartate-semialdehyde dehydrogenase; ?	Serebrijski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," *J. Bacteriol.*, 177(24):7255-7260 (1995)
[redacted]2929	proA	Gamma-glutamyl phosphate reductase	Serebrijski, I. et al. "Multicopy suppression by asd gene and osmotic stress-dependent complementation by heterologous proA in proA mutants," *J. Bacteriol.*, 177(24):7255-7260 (1995)
X84257	16S rDNA	16S ribosomal RNA	Pascual, C. et al. "Phylogenetic analysis of the genus Corynebacterium based on 16S rRNA gene sequences," *Int. J. Syst. Bacteriol.*, 45(4):724-728 (1995)
X85965	aroP; dapE	Aromatic amino acid permease; ?	Wehrmann, A. et al. "Functional analysis of sequences adjacent to dapE of Corynebacterium glutamicumproline reveals the presence of aroP, which encodes the aromatic amino acid transporter," *J. Bacteriol.*, 177(20):5991-5993 (1995)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X86157	argB; argC; argD; argF; argJ	Acetylglutamate kinase; N-acetyl-gamma-glutamyl-phosphate reductase; acetylornithine aminotransferase; ornithine carbamoyltransferase; glutamate N-acetyltransferase	Sakanyan, V. et al. "Genes and enzymes of the acetyl cycle of arginine biosynthesis in Corynebacterium glutamicum: enzyme evolution in the early steps of the arginine pathway," *Microbiology*, 142:99-108 (1996)
[redacted]89084	pta; ackA	Phosphate acetyltransferase; acetate kinase	Reinscheid, D.J. et al. "Cloning, sequence analysis, expression and inactivation of the Corynebacterium glutamicum pta-ack operon encoding phosphotransacetylase and acetate kinase," *Microbiology*, 145:503-513 (1999)
X89850	attB	Attachment site	Le Marrec, C. et al. "Genetic characterization of site-specific integration functions of phi AAU2 infecting "Arthrobacter aureus C70," *J. Bacteriol.*, 178(7):1996-2004 (1996)
X90356		Promoter fragment F1	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90357		Promoter fragment F2	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90358		Promoter fragment F10	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90359		Promoter fragment F13	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
[redacted]0360		Promoter fragment F22	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90361		Promoter fragment F34	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)
X90362		Promoter fragment F37	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology*, 142:1297-1309 (1996)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X90363		Promoter fragment F45	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
X90364		Promoter fragment F64	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
[redacted]0365		Promoter fragment F75	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
X90366		Promoter fragment PF101	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
X90367		Promoter fragment PF104	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
X90368		Promoter fragment PF109	Patek, M. et al. "Promoters from Corynebacterium glutamicum: cloning, molecular analysis and search for a consensus motif," *Microbiology,* 142:1297-1309 (1996)
X93513	amt	Ammonium transport system	Siewe, R.M. et al. "Functional and genetic characterization of the (methyl) ammonium uptake carrier of Corynebacterium glutamicum," *J. Biol. Chem.,* 271(10):5398-5403 (1996)
X93514	betP	Glycine betaine transport system	Peter, H. et al. "Isolation, characterization, and expression of the Corynebacterium glutamicum betP gene, encoding the transport system for the compatible solute glycine betaine," *J. Bacteriol.,* 178(17):5229-5234 (1996)
[redacted]5649	orf4		Patek, M. et al. "Identification and transcriptional analysis of the dapB-ORF2-dapA-ORF4 operon of Corynebacterium glutamicum, encoding two enzymes involved in L-lysine synthesis," *Biotechnol. Lett.,* 19:1113-1117 (1997)
X96471	lysE; lysG	Lysine exporter protein; Lysine export regulator protein	Vrljic, M. et al. "A new type of transporter with a new type of cellular function: L-lysine export from Corynebacterium glutamicum," *Mol. Microbiol.,* 22(5):815-826 (1996)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
X96580	panB; panC; xylB	3-methyl-2-oxobutanoate hydroxymethyltransferase; pantoate-beta-alanine ligase; xylulokinase	Sahm, H. et al. "D-pantothenate synthesis in Corynebacterium glutamicum and use of panBC and genes encoding L-valine synthesis for D-pantothenate overproduction," *Appl. Environ. Microbiol.*, 65(5):1973-1979 (1999)
X96962		Insertion sequence IS1207 and transposase	
X99289		Elongation factor P	Ramos, A. et al. "Cloning, sequencing and expression of the gene encoding elongation factor P in the amino-acid producer Brevibacterium lactofermentum (Corynebacterium glutamicum ATCC 13869)," *Gene*, 198:217-222 (1997)
[redacted]0140	thrB	Homoserine kinase	Mateos, L.M. et al. "Nucleotide sequence of the homoserine kinase (thrB) gene of the Brevibacterium lactofermentum," *Nucleic Acids Res.*, 15(9):3922 (1987)
Y00151	ddh	Meso-diaminopimelate D-dehydrogenase (EC 1.4.1.16)	Ishino, S. et al. "Nucleotide sequence of the meso-diaminopimelate D-dehydrogenase gene from Corynebacterium glutamicum," *Nucleic Acids Res.*, 15(9):3917 (1987)
Y00476	thrA	Homoserine dehydrogenase	Mateos, L.M. et al. "Nucleotide sequence of the homoserine dehydrogenase (thrA) gene of the Brevibacterium lactofermentum," *Nucleic Acids Res.*, 15(24):10598 (1987)
Y00546	hom; thrB	Homoserine dehydrogenase; homoserine kinase	Peoples, O.P. et al. "Nucleotide sequence and fine structural analysis of the Corynebacterium glutamicum hom-thrB operon," *Mol. Microbiol.*, 2(1):63-72 (1988)
Y08964	murC; ftsQ/divD; ftsZ	UPD-N-acetylmuramate-alanine ligase; division initiation protein or cell division protein; cell division protein	Honrubia, M.P. et al. "Identification, characterization, and chromosomal organization of the ftsZ gene from Brevibacterium lactofermentum," *Mol. Gen. Genet.*, 259(1):97-104 (1998)
Y09163	putP	High affinity proline transport system	Peter, H. et al. "Isolation of the putP gene of Corynebacterium glutamicumproline and characterization of a low-affinity uptake system for compatible solutes," *Arch. Microbiol.*, 168(2):143-151 (1997)
[redacted]9548	pyc	Pyruvate carboxylase	Peters-Wendisch, P.G. et al. "Pyruvate carboxylase from Corynebacterium glutamicum: characterization, expression and inactivation of the pyc gene," *Microbiology*, 144:915-927 (1998)
Y09578	leuB	3-isopropylmalate dehydrogenase	Patek, M. et al. "Analysis of the leuB gene from Corynebacterium glutamicum," *Appl. Microbiol. Biotechnol.*, 50(1):42-47 (1998)
Y12472		Attachment site bacteriophage Phi-16	Moreau, S. et al. "Site-specific integration of corynephage Phi-16: The construction of an integration vector," *Microbiol.*, 145:539-548 (1999)

GenBank™ Accession No.	Gene Name	Gene Function	Reference
Y12537	proP	Proline/ectoine uptake system protein	Peter, H. et al. "Corynebacterium glutamicum is equipped with four secondary carriers for compatible solutes: Identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP," *J. Bacteriol.*, 180(22):6005-6012 (1998)
Y13221	glnA	Glutamine synthetase I	Jakoby, M. et al. "Isolation of Corynebacterium glutamicum glnA gene encoding glutamine synthetase I," *FEMS Microbiol. Lett.*, 154(1):81-88 (1997)
[redacted]16642	lpd	Dihydrolipoamide dehydrogenase	
[redacted]8059		Attachment site Corynephage 304L	Moreau, S. et al. "Analysis of the integration functions of φ304L: An integrase module among corynephages," *Virology*, 255(1):150-159 (1999)
Z21501	argS; lysA	Arginyl-tRNA synthetase; diaminopimelate decarboxylase (partial)	Oguiza, J.A. et al. "A gene encoding arginyl-tRNA synthetase is located in the upstream region of the lysA gene in Brevibacterium lactofermentum: Regulation of argS-lysA cluster expression by arginine," *J. Bacteriol.*,175(22):7356-7362 (1993)
Z21502	dapA; dapB	Dihydrodipicolinate synthase; dihydrodipicolinate reductase	Pisabarro, A. et al. "A cluster of three genes (dapA, orf2, and dapB) of Brevibacterium lactofermentum encodes dihydrodipicolinate reductase, and a third polypeptide of unknown function," *J. Bacteriol.*, 175(9):2743-2749 (1993)
Z29563	thrC	Threonine synthase	Malumbres, M. et al. "Analysis and expression of the thrC gene of the encoded threonine synthase," *Appl. Environ. Microbiol.*, 60(7)2209-2219 (1994)
Z46753	16S rDNA	Gene for 16S ribosomal RNA	
Z49822	sigA	SigA sigma factor	Oguiza, J.A. et al "Multiple sigma factor genes in Brevibacterium lactofermentum: Characterization of sigA and sigB," *J. Bacteriol.*, 178(2):550-553 (1996)
[redacted]9823	galE; dtxR	Catalytic activity UDP-galactose 4-epimerase; diphtheria toxin regulatory protein	Oguiza, J.A. et al "The galE gene encoding the UDP-galactose 4-epimerase of Brevibacterium lactofermentum is coupled transcriptionally to the dmdR gene," *Gene*, 177:103-107 (1996)
Z49824	orf1; sigB	?; SigB sigma factor	Oguiza, J.A. et al "Multiple sigma factor genes in Brevibacterium lactofermentum: Characterization of sigA and sigB," *J. Bacteriol.*, 178(2):550-553 (1996)
Z66534		Transposase	Correia, A. et al. "Cloning and characterization of an IS-like element present in the genome of Brevibacterium lactofermentum ATCC 13869," *Gene*, 170(1):91-94 (1996)

[1] A sequence for this gene was published in the indicated reference. However, the sequence obtained by the inventors of the present application is significantly longer than the published version. It is believed that the published version relied on an incorrect start codon, and thus represents only a fragment of the actual coding region.

TABLE 3: Corynebacterium and Brevibacterium Strains Which May be Used in the Practice of the Invention

Genus	species	ATCC	FERM	NRRL	CECT	NCIMB	CBS	NCTC	DSMZ
Brevibacterium	ammoniagenes	21054							
Brevibacterium	ammoniagenes	19350							
Brevibacterium	ammoniagenes	19351							
Brevibacterium	ammoniagenes	19352							
Brevibacterium	ammoniagenes	19353							
Brevibacterium	ammoniagenes	19354							
Brevibacterium	ammoniagenes	19355							
Brevibacterium	ammoniagenes	19356							
Brevibacterium	ammoniagenes	21055							
Brevibacterium	ammoniagenes	21077							
Brevibacterium	ammoniagenes	21553							
Brevibacterium	ammoniagenes	21580							
Brevibacterium	ammoniagenes	39101							
Brevibacterium	butanicum	21196							
Brevibacterium	divaricatum	21792	P928						
Brevibacterium	flavum	21474							
Brevibacterium	flavum	21129							
Brevibacterium	flavum	21518							
Brevibacterium	flavum			B11474					
Brevibacterium	flavum			B11472					
Brevibacterium	flavum	21127							
Brevibacterium	flavum	21128							
Brevibacterium	flavum	21427							
Brevibacterium	flavum	21475							
Brevibacterium	flavum	21517							
Brevibacterium	flavum	21528							
Brevibacterium	flavum	21529							

Brevibacterium	flavum			B11477					
Brevibacterium	flavum			B11478					
Brevibacterium	flavum	21127							
Brevibacterium	flavum			B11474					
Brevibacterium	healii	15527							
Brevibacterium	ketoglutamicum	21004							
Brevibacterium	ketoglutamicum	21089							
Brevibacterium	ketosoreductum	21914							
Brevibacterium	lactofermentum				70				
Brevibacterium	lactofermentum				74				
Brevibacterium	lactofermentum				77				
Brevibacterium	lactofermentum	21798							
Brevibacterium	lactofermentum	21799							
Brevibacterium	lactofermentum	21800							
Brevibacterium	lactofermentum	21801							
Brevibacterium	lactofermentum			B11470					
Brevibacterium	lactofermentum			B11471					
Brevibacterium	lactofermentum	21086							
Brevibacterium	lactofermentum	21420							
Brevibacterium	lactofermentum	21086							
Brevibacterium	lactofermentum	31269							
Brevibacterium	linens	9174							
Brevibacterium	linens	19391							
Brevibacterium	linens	8377							
Brevibacterium	paraffinolyticum					11160			
Brevibacterium	spec.						717.73		
Brevibacterium	spec.						717.73		
Brevibacterium	spec.	14604							
Brevibacterium	spec.	21860							
Brevibacterium	spec.	21864							
Brevibacterium	spec.	21865							

09602839 . 062300

Brevibacterium	spec.	21866							
Brevibacterium	spec.	19240							
Corynebacterium	acetoacidophilum	21476							
Corynebacterium	acetoacidophilum	13870							
Corynebacterium	acetoglutamicum			B11473					
Corynebacterium	acetoglutamicum			B11475					
Corynebacterium	acetoglutamicum	15806							
Corynebacterium	acetoglutamicum	21491							
Corynebacterium	acetoglutamicum	31270							
Corynebacterium	acetophilum			B3671					
Corynebacterium	ammoniagenes	6872						2399	
Corynebacterium	ammoniagenes	15511							
Corynebacterium	fujiokense	21496							
Corynebacterium	glutamicum	14067							
Corynebacterium	glutamicum	39137							
Corynebacterium	glutamicum	21254							
Corynebacterium	glutamicum	21255							
Corynebacterium	glutamicum	31830							
Corynebacterium	glutamicum	13032							
Corynebacterium	glutamicum	14305							
Corynebacterium	glutamicum	15455							
Corynebacterium	glutamicum	13058							
Corynebacterium	glutamicum	13059							
Corynebacterium	glutamicum	13060							
Corynebacterium	glutamicum	21492							
Corynebacterium	glutamicum	21513							
Corynebacterium	glutamicum	21526							
Corynebacterium	glutamicum	21543							
Corynebacterium	glutamicum	13287							
Corynebacterium	glutamicum	21851							
Corynebacterium	glutamicum	21253							

0960283 9.062300

Corynebacterium	glutamicum	21514							
Corynebacterium	glutamicum	21516							
Corynebacterium	glutamicum	21299							
Corynebacterium	glutamicum	21300							
Corynebacterium	glutamicum	39684							
Corynebacterium	glutamicum	21488							
Corynebacterium	glutamicum	21649							
Corynebacterium	glutamicum	21650							
Corynebacterium	glutamicum	19223							
Corynebacterium	glutamicum	13869							
Corynebacterium	glutamicum	21157							
Corynebacterium	glutamicum	21158							
Corynebacterium	glutamicum	21159							
Corynebacterium	glutamicum	21355							
Corynebacterium	glutamicum	31808							
Corynebacterium	glutamicum	21674							
Corynebacterium	glutamicum	21562							
Corynebacterium	glutamicum	21563							
Corynebacterium	glutamicum	21564							
Corynebacterium	glutamicum	21565							
Corynebacterium	glutamicum	21566							
Corynebacterium	glutamicum	21567							
Corynebacterium	glutamicum	21568							
Corynebacterium	glutamicum	21569							
Corynebacterium	glutamicum	21570							
Corynebacterium	glutamicum	21571							
Corynebacterium	glutamicum	21572							
Corynebacterium	glutamicum	21573							
Corynebacterium	glutamicum	21579							
Corynebacterium	glutamicum	19049							
Corynebacterium	glutamicum	19050							

Corynebacterium	glutamicum	19051							
Corynebacterium	glutamicum	19052							
Corynebacterium	glutamicum	19053							
Corynebacterium	glutamicum	19054							
Corynebacterium	glutamicum	19055							
Corynebacterium	glutamicum	19056							
Corynebacterium	glutamicum	19057							
Corynebacterium	glutamicum	19058							
Corynebacterium	glutamicum	19059							
Corynebacterium	glutamicum	19060							
Corynebacterium	glutamicum	19185							
Corynebacterium	glutamicum	13286							
Corynebacterium	glutamicum	21515							
Corynebacterium	glutamicum	21527							
Corynebacterium	glutamicum	21544							
Corynebacterium	glutamicum	21492							
Corynebacterium	glutamicum			B8183					
Corynebacterium	glutamicum			B8182					
Corynebacterium	glutamicum			B12416					
Corynebacterium	glutamicum			B12417					
Corynebacterium	glutamicum			B12418					
Corynebacterium	glutamicum			B11476					
Corynebacterium	glutamicum	21608							
Corynebacterium	lilium		P973						
Corynebacterium	nitrilophilus	21419				11594			
Corynebacterium	spec.		P4445						
Corynebacterium	spec.		P4446						
Corynebacterium	spec.	31088							
Corynebacterium	spec.	31089							
Corynebacterium	spec.	31090							
Corynebacterium	spec.	31090							

Corynebacterium	spec.	31090							
Corynebacterium	spec.	15954							20145
Corynebacterium	spec.	21857							
Corynebacterium	spec.	21862							
Corynebacterium	spec.	21863							

ATCC: American Type Culture Collection, Rockville, MD, USA

FERM: Fermentation Research Institute, Chiba, Japan

NRRL: ARS Culture Collection, Northern Regional Research Laboratory, Peoria, IL, USA

CECT: Coleccion Espanola de Cultivos Tipo, Valencia, Spain

NCIMB: National Collection of Industrial and Marine Bacteria Ltd., Aberdeen, UK

CBS: Centraalbureau voor Schimmelcultures, Baarn, NL

NCTC: National Collection of Type Cultures, London, UK

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

For reference see Sugawara, H. et al. (1993) World directory of collections of cultures of microorganisms: Bacteria, fungi and yeasts (4th edn), World federation for culture collections world data center on microorganisms, Saimata, Japen.

TABLE 4: ALIGNMENT RESULTS

ID #	length (NT)	Genbank Hit	Length	Accession	Name of Genbank Hit	Source of Genbank Hit	% homology (GAP)	Date of Deposit
rxa00005	1731	GB_BA1:CGGLNA	3686	Y13221	Corynebacterium glutamicum glnA gene.	Corynebacterium glutamicum	37,555	28-Aug-97
		GB_BA1:CGPROMF45	60	X90363	C.glutamicum DNA for promoter fragment F45.	Corynebacterium glutamicum	100,000	4-Nov-96
		GB_BA1:CGGLNA	3686	Y13221	Corynebacterium glutamicum glnA gene.	Corynebacterium glutamicum	37,251	28-Aug-97
rxa00011	480	GB_BA1:D86821	5585	D86821	Streptomyces coelicolor DNA for PkaA, PkaB and PrfB, complete cds.	Streptomyces coelicolor	69,729	7-Feb-99
		GB_BA1:MTCY164	39150	Z95150	Mycobacterium tuberculosis H37Rv complete genome; segment 135/162.	Mycobacterium tuberculosis	35,639	19-Jun-98
		GB_BA1:MLCB1779	43254	Z98271	Mycobacterium leprae cosmid B1779.	Mycobacterium leprae	37,555	8-Aug-97
rxa00012	999	GB_BA1:D86821	5585	D86821	Streptomyces coelicolor DNA for PkaA, PkaB and PrfB, complete cds.	Streptomyces coelicolor	63,089	7-Feb-99
		GB_BA1:MTCY164	39150	Z95150	Mycobacterium tuberculosis H37Rv complete genome; segment 135/162.	Mycobacterium tuberculosis	38,985	19-Jun-98
		GB_BA1:MLCB1779	43254	Z98271	Mycobacterium leprae cosmid B1779.	Mycobacterium leprae	37,448	8-Aug-97
rxa00016	1017	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	82,891	9-Aug-95
		GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	83,201	24-Jun-98
		GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	83,201	29-Sep-99
rxa00017	417	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	78,947	9-Aug-95
		GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	77,895	24-Jun-98
		GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	77,895	29-Sep-99
rxa00019	1983	GB_PR3:HSN20A6	30875	Z69713	Human DNA sequence from cosmid cN20A6, on chromosome 22 contains STS,	Homo sapiens	37,596	23-Nov-99
		GB_HTG3:AC011577	151996	AC011577	Homo sapiens clone 12_P_19, LOW-PASS SEQUENCE SAMPLING.	Homo sapiens	34,506	07-OCT-1999
		GB_EST37:AW000587	470	AW000587	614056A09.x1 614 - root cDNA library from Walbot Lab Zea mays cDNA, mRNA sequence.	Zea mays	41,578	8-Sep-99
rxa00046	819	GB_EST17:C73675	391	C73675	C73675 Rice panicle (longer than 10cm) Oryza sativa cDNA clone E20126_2A, mRNA sequence.	Oryza sativa	42,014	23-Sep-97
		GB_EST31:AI704169	275	AI704169	UI-R-AC0-yi-d-08-0-UI.s1 UI-R-AC0 Rattus norvegicus cDNA clone UI-R-AC0-yi-d-08-0-UI 3', mRNA sequence.	Rattus norvegicus	38,182	3-Jun-99
		GB_EST35:AI846250	390	AI846250	UI-M-AK1-aez-b-06-0-UI.s1 NIH_BMAP_MHY_N Mus musculus cDNA clone UI-M-AK1-aez-b-06-0-UI 3', mRNA sequence.	Mus musculus	34,872	15-Jul-99
rxa00053	516	GB_PL2:AF072675	3127	AF072675	Kluyveromyces lactis Hap4p (HAP4) gene, complete cds.	Kluyveromyces lactis	36,914	13-MAR-1999
		GB_VI:AB010886	3387	AB010886	Cydia pomonella granulovirus genes for chitinase and cathepsin, complete cds.	Cydia pomonella granulovirus	35,375	13-Feb-99
		GB_PR2:HS1177I5	96256	AL022315	Human DNA sequence from clone 1177I5 on chromosome 22q13.1. Contains part of a putative novel gene, the gene for serum constituent protein MSE55 downstream of a putative CpG island and the LGALS2 gene for Lectin, Galactose-binding, soluble, 2 (Galectin 2, S-Lac Lectin 2, HL14). Contains ESTs and GSSs, complete sequence.	Homo sapiens	36,884	23-Nov-99
rxa00057	222	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	61,261	9-Aug-95
		GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	66,512	29-Sep-99
		GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	66,512	24-Jun-98
rxa00069	1506	GB_BA1:MTCY27	27548	Z95208	Mycobacterium tuberculosis H37Rv complete genome; segment 104/162.	Mycobacterium tuberculosis	38,029	17-Jun-98
		GB_BA1:MSGB1229CS	30670	L78812	Mycobacterium leprae cosmid B1229 DNA sequence.	Mycobacterium leprae	64,940	15-Jun-96
		GB_BA1:MSGB998CS	10000	L78829	Mycobacterium leprae cosmid B998 DNA sequence.	Mycobacterium leprae	64,940	15-Jun-96
rxa00102	891	GB_PR3:HS45P21	170001	AL021917	Human DNA sequence from clone 45P21 on chromosome 6p21.3-22.2 Contains butyrophilins (BTF3, BTF5, BTF2, BTF4), EST, STS, complete sequence.	Homo sapiens	37,882	23-Nov-99
		GB_PR3:AC005330	40607	AC005330	Homo sapiens chromosome 19, cosmid R34047, complete sequence.	Homo sapiens	35,666	28-Jul-98

TABLE 4: ALIGNMENT RESULTS

	GB_GSS4:AQ677431	502	AQ677431	HS_5529_A2_C01_T7A RPCI-11 Human Male BAC Library Homo sapiens genomic clone Plate=1105 Col=2 Row=E, genomic survey sequence.	Homo sapiens	37,000	25-Jun-99
rxa00107 360	GB_PL2:AC007153	103223	AC007153	Arabidopsis thaliana chromosome I BAC F3F20 genomic sequence, complete sequence.	Arabidopsis thaliana	33,427	17-MAY-1999
	GB_BA2:PPU89363	4642	U89363	Pseudomonas putida P38K, amidase, nitrile hydratase alpha subunit, nitrile hydratase beta subunit, and P14K genes, complete cds.	Pseudomonas putida	40,988	2-Jun-98
	GB_PAT:AR041193	1440	AR041193	Sequence 17 from patent US 5811286.	Unknown.	40,988	29-Sep-99
rxa00125 888	EM_PAT:E09053	2538	E09053	gDNA encoding secA protein.	Corynebacterium glutamicum	94,028	07-OCT-1997 (Rel. 52, Created)
	GB_BA1:MSU66081	2968	U66081	Mycobacterium smegmatis SecA (SecA) gene, complete cds.	Mycobacterium smegmatis	71,216	28-Aug-96
	GB_BA2:SLU21192	4006	U21192	Streptomyces lividans SecA (secA) gene, complete cds.	Streptomyces lividans	63,472	3-Sep-96
	GB_BA1:BLELONP	738	X99289	B.lactofermentum gene encoding elongation factor P.	Corynebacterium glutamicum	98,331	1-Nov-97
rxa00138 684	GB_BA1:MTCY159	33818	Z83863	Mycobacterium tuberculosis H37Rv complete genome; segment 111/162.	Mycobacterium tuberculosis	37,946	17-Jun-98
	GB_BA1:MSGB937CS	38914	L78820	Mycobacterium leprae cosmid B937 DNA sequence.	Mycobacterium leprae	62,261	15-Jun-96
rxa00172 735	GB_EST28:AI484755	572	AI484755	EST243016 tomato ovary, TAMU Lycopersicon esculentum cDNA clone cLED3O13, mRNA sequence.	Lycopersicon esculentum	39,171	29-Jun-99
	GB_EST28:AI486041	610	AI486041	EST244362 tomato ovary, TAMU Lycopersicon esculentum cDNA clone cLED2K7, mRNA sequence.	Lycopersicon esculentum	46,452	29-Jun-99
	GB_PR3:HS396D17	152592	AL008634	Human DNA sequence from clone 396D17 on chromosome 1p33-35.3 Contains EST, STS, GSS, complete sequence.	Homo sapiens	33,060	23-Nov-99
	GB_BA1:MTCY50	36030	Z77137	Mycobacterium tuberculosis H37Rv complete genome; segment 55/162.	Mycobacterium tuberculosis	47,823	17-Jun-98
rxa00184 1296	GB_BA1:AB013492	18497	AB013492	Bacillus halodurans C-125 genomic DNA, 9A/3S' fragment, clone ALBAC001.	Bacillus halodurans	39,234	3-Aug-99
	GB_PR3:AC005738	134506	AC005738	Homo sapiens chromosome 5, BAC clone 7g12 (LBNL H126), complete sequence.	Homo sapiens	37,127	20-OCT-1998
	GB_BA1:MTV012	70287	AL021287	Mycobacterium tuberculosis H37Rv complete genome; segment 132/162.	Mycobacterium tuberculosis	37,632	23-Jun-99
rxa00209 1614	GB_BA1:MLCB637	44882	Z99263	Mycobacterium leprae cosmid B637.	Mycobacterium leprae	65,785	17-Sep-97
	GB_BA1:SC8D9	38681	AL035569	Streptomyces coelicolor cosmid 8D9.	Streptomyces coelicolor	63,795	26-Feb-99
	GB_PL1:MGR7031	103	AJ007031	Mycosphaerella graminicola microsatellite ST1A2 DNA.	Mycosphaerella graminicola	45,545	3-Aug-98
rxa00210 420	GB_HTG1:CEY48G10_4	110000	AL021450	Caenorhabditis elegans chromosome I clone Y48G10, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	37,101	29-Jul-99
	GB_HTG1:CEY48G10_4	110000	AL021450	Caenorhabditis elegans chromosome I clone Y48G10, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	37,101	29-Jul-99
	GB_BA1:MTV012	70287	AL021287	Mycobacterium tuberculosis H37Rv complete genome; segment 132/162.	Mycobacterium tuberculosis	35,122	23-Jun-99
rxa00217 1218	GB_HTG2:AC008092	88749	AC008092	Drosophila melanogaster chromosome 3 clone BACR22F22 (D824) RPCI-98 22.F.22 map 84D-84D strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 53 unordered pieces.	Drosophila melanogaster	33,001	2-Aug-99
	GB_HTG2:AC008092	88749	AC008092	Drosophila melanogaster chromosome 3 clone BACR22F22 (D824) RPCI-98 22.F.22 map 84D-84D strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 53 unordered pieces.	Drosophila melanogaster	33,001	2-Aug-99
	GB_BA1:LPLLDHE	1651	X70926	L.plantarum gene for l-lactate dehydrogenase.	Lactobacillus plantarum	37,294	17-Feb-94
rxa00227 921	GB_GSS9:AQ158656	731	AQ158656	nbxb0011N08f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0011N08f, genomic survey sequence.	Oryza sativa	39,041	12-Sep-98
	GB_BA1:LPLLDHE	1651	X70926	L.plantarum gene for l-lactate dehydrogenase.	Lactobacillus plantarum	34,947	17-Feb-94

TABLE 4: ALIGNMENT RESULTS

rxa00265 573	GB_PR4:AC007368	94024	AC007368	Homo sapiens 12q24.2 PAC RPCI4-809F18 (Roswell Park Cancer Institute Human PAC Library) complete sequence.	Homo sapiens	40,037	31-Jul-99
	GB_PR4:AC007368	94024	AC007368	Homo sapiens 12q24.2 PAC RPCI4-809F18 (Roswell Park Cancer Institute Human PAC Library) complete sequence.	Homo sapiens	36,121	31-Jul-99
rxa00280 624	GB_EST29:AI588595	532	AI588595	fb97b04.y1 Zebrafish WashU MPIMG EST Danio rerio cDNA 5' similar to WP:F32D8.4 CE05783 LACTATE DEHYDROGENASE ;, mRNA sequence.	Danio rerio	34,242	21-Apr-99
	GB_VI:D78362	1593	D78362	Rotavirus sp. mRNA for nonstructural protein 1, complete cds.	Rotavirus sp.	35,217	22-Jan-98
	GB_EST29:AI588595	532	AI588595	fb97b04.y1 Zebrafish WashU MPIMG EST Danio rerio cDNA 5' similar to WP:F32D8.4 CE05783 LACTATE DEHYDROGENASE ;, mRNA sequence.	Danio rerio	36,118	21-Apr-99
rxa00314 1503	GB_PAT:AR008345	1344	AR008345	Sequence 1 from patent US 5753480.	Unknown.	50,783	04-DEC-1998
	GB_BA1:ABIPDC	4933	X99587	A.brasilense ipdC, gltX & cysS genes.	Azospirillum brasilense	37,244	9-Jan-98
	GB_PAT:AR008346	333	AR008346	Sequence 3 from patent US 5753480.	Unknown.	64,545	04-DEC-1998
rxa00331 480	GB_BA1:CGTHRC	3120	X56037	Corynebacterium glutamicum thrC gene for threonine synthase (EC4.2.99.2).	Corynebacterium glutamicum	40,393	17-Jun-97
	GB_PAT:I09078	3146	I09078	Sequence 4 from Patent WO 8809819.	Unknown.	38,462	02-DEC-1994
	GB_BA1:SAY14370	7791	Y14370	Staphylococcus aureus RF3, murE, ypfP genes.	Staphylococcus aureus	34,526	24-Jun-98
rxa00333 657	GB_PR3:AC004788	39436	AC004788	Homo sapiens chromosome 7 clone UWGC:g1564a327 from 7p14-15, complete sequence.	Homo sapiens	37,618	2-Jun-98
	GB_PR3:AC004788	39436	AC004788	Homo sapiens chromosome 7 clone UWGC:g1564a327 from 7p14-15, complete sequence.	Homo sapiens	34,169	2-Jun-98
rxa00454 1416	GB_BA2:AE000147	10577	AE000147	Escherichia coli K-12 MG1655 section 37 of 400 of the complete genome.	Escherichia coli	48,925	12-Nov-98
	GB_PR4:DJ270M14	192126	AF107885	Homo sapiens chromosome 14q24.3 clone BAC270M14 transforming growth factor beta 3 (TGF-beta 3) gene, complete cds; and unknown genes.	Homo sapiens	36,043	14-Jul-99
	GB_BA1:ECOTGT	1823	M63939	E.coli tRNA-guanine-transglycosylase (tgt) gene, complete cds.	Escherichia coli	48,925	26-APR-199
	GB_BA1:SC4G2	30590	AL031371	Streptomyces coelicolor cosmid 4G2.	Streptomyces coelicolor	34,836	5-Sep-98
rxa00458 736	GB_BA2:AF024619	4038	AF024619	Pseudomonas fluorescens hybrid histidine kinase homolog (styS) and response regulatory protein (styR) genes, complete cds.	Pseudomonas fluorescens	39,251	23-MAR-1998
	GB_BA1:SC4G2	30590	AL031371	Streptomyces coelicolor cosmid 4G2.	Streptomyces coelicolor	40,196	5-Sep-98
rxa00484 1203	GB_PL1:AB012627	3019	AB012627	Adiantum capillus-veneris CRY2 mRNA for blue-light photoreceptor,complete cds.	Adiantum capillus-veneris	43,959	5-Feb-99
	GB_PL1:AB012630	4098	AB012630	Adiantum capillus-veneris CRY2 gene for blue-light photoreceptor, complete cds.	Adiantum capillus-veneris	39,765	5-Feb-99
	GB_PL1:YSCF6552A	20383	D31600	Saccharomyces cerevisiae chromosome VI phage 6552.	Saccharomyces cerevisiae	37,133	7-Feb-99
rxa00495 687	GB_HTG3:AC008853	54169	AC008853	Homo sapiens chromosome 5 clone CITB-H1_2176P21, *** SEQUENCING IN PROGRESS ***, 66 unordered pieces.	Homo sapiens	36,471	3-Aug-99
	GB_HTG3:AC008853	54169	AC008853	Homo sapiens chromosome 5 clone CITB-H1_2176P21, *** SEQUENCING IN PROGRESS ***, 66 unordered pieces.	Homo sapiens	36,471	3-Aug-99
	GB_HTG3:AC008853	54169	AC008853	Homo sapiens chromosome 5 clone CITB-H1_2176P21, *** SEQUENCING IN PROGRESS ***, 66 unordered pieces.	Homo sapiens	36,090	3-Aug-99
rxa00532 608	GB_BA1:ECR751	5499	X54458	E. coli plasmid R751 traF (5'end), traG, traH, traI, traJ, traK and traL (5'end) genes of the transfer region.	Escherichia coli	38,992	18-Nov-93

09602839 .062300

TABLE 4: ALIGNMENT RESULTS

		GB_BA2:EAU67194	53339	U67194	Enterobacter aerogenes plasmid R751, complete plasmid sequence.	Enterobacter aerogenes	38,992	19-OCT-1998
		GB_BA1:D83237	1626	D83237	Rhodococcus erythropolis DNA for catechol 1,2-dioxgenase, complete cds.	Rhodococcus erythropolis	37,232	1-Sep-99
rxa00539	600	GB_PL2:AF053311	1110	AF053311	Zantedeschia aethiopica glutathione peroxidase (gpx) mRNA, nuclear gene encoding chloroplast protein, complete cds.	Zantedeschia aethiopica	48,552	20-Nov-98
		GB_PL2:AF053311	1110	AF053311	Zantedeschia aethiopica glutathione peroxidase (gpx) mRNA, nuclear gene encoding chloroplast protein, complete cds.	Zantedeschia aethiopica	36,301	20-Nov-98
rxa00568	1470	GB_PAT:I92046	2203	I92046	Sequence 13 from patent US 5726299.	Unknown.	37,129	01-DEC-1998
		GB_PAT:I78757	2203	I78757	Sequence 13 from patent US 5693781.	Unknown.	37,129	3-Apr-98
		GB_PR4:AC005042	192218	AC005042	Homo sapiens clone NH0552E01, complete sequence.	Homo sapiens	37,672	14-Jan-99
rxa00588	645	GB_BA1:MTV017	67200	AL021897	Mycobacterium tuberculosis H37Rv complete genome; segment 48/162.	Mycobacterium tuberculosis	36,150	24-Jun-99
		GB_BA1:PAU81259	7285	U81259	Pseudomonas aeruginosa dihydrodipicolinate reductase (dapB) gene, partial cds, carbamoylphosphate synthetase small subunit (carA) and carbamoylphosphate synthetase large subunit (carB) genes, complete cds, and FtsJ homolog (ftsJ) gene, partial cds.	Pseudomonas aeruginosa	45,483	23-DEC-1996
		GB_IN2:AC005643	80389	AC005643	Drosophila melanogaster, chromosome 2R, region 50C5-50C8, P1 clone DS02972, complete sequence.	Drosophila melanogaster	40,705	15-DEC-1998
		GB_BA1:MLCB1222	34714	AL049491	Mycobacterium leprae cosmid B1222.	Mycobacterium leprae	40,549	27-Aug-99
rxa00677	339	GB_BA1:MBU15140	2136	U15140	Mycobacterium bovis ribosomal proteins IF-1 (infA), L36 (rpmJ), S13 (rpsM) and S11 (rpsK) genes, complete cds, and S4 (rpsD) gene, partial cds.	Mycobacterium bovis	64,881	28-OCT-1996
		GB_BA1:MTY13E12	43401	Z95390	Mycobacterium tuberculosis H37Rv complete genome; segment 147/162.	Mycobacterium tuberculosis	41,896	17-Jun-98
rxa00687	1443	GB_BA1:BRLSECY	1516	D14162	Brevibacterium flavum gene for SecY protein (complete cds) and gene for adenylate kinase (partial cds).	Corynebacterium glutamicum	98,436	3-Feb-99
		GB_PAT:E07701	1323	E07701	Brevibacterium secY gene.	Corynebacterium glutamicum	98,262	29-Sep-97
		GB_BA1:MTV041	28826	AL021958	Mycobacterium tuberculosis H37Rv complete genome; segment 35/162.	Mycobacterium tuberculosis	60,724	17-Jun-98
rxa00753	1704	GB_EST17:C61980	216	C61980	C61980 Yuji Kohara unpublished cDNA Caenorhabditis elegans cDNA clone yk272b4 5', mRNA sequence.	Caenorhabditis elegans	43,030	22-Sep-97
		GB_RO:MMANT12	5141	X01815	Mouse gene for H-2K(d) antigen.	Mus musculus	37,317	03-OCT-1997
		GB_PR4:AC003001	101981	AC003001	Homo sapiens chromosome X, clone HRPC928E24, complete sequence.	Homo sapiens	34,127	6-Feb-99
rxa00824	681	GB_PL2:ATFCA0	200576	Z97335	Arabidopsis thaliana DNA chromosome 4, ESSA I FCA contig fragment No. 0.	Arabidopsis thaliana	36,527	28-Jun-99
		GB_PR4:AC006443	210636	AC006443	Homo sapiens chromosome 9, clone hRPK.494_N_15, complete sequence.	Homo sapiens	38,401	30-Jan-99
		GB_PR4:AC006443	210636	AC006443	Homo sapiens chromosome 9, clone hRPK.494_N_15, complete sequence.	Homo sapiens	34,027	30-Jan-99
rxa00896	702	GB_GSS12:AQ403148	432	AQ403148	HS_5052_A2_F07_SP6E RPCI-11 Human Male BAC Library Homo sapiens genomic clone Plate=628 Col=14 Row=K, genomic survey sequence.	Homo sapiens	41,371	13-MAR-1999
		GB_HTG6:AC009921	184689	AC009921	Homo sapiens clone RP11-115O18, WORKING DRAFT SEQUENCE, 17 unordered pieces.	Homo sapiens	37,223	03-DEC-1999
		GB_HTG6:AC009921	184689	AC009921	Homo sapiens clone RP11-115O18, WORKING DRAFT SEQUENCE, 17 unordered pieces.	Homo sapiens	38,438	03-DEC-1999
rxa00927	1212	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	36,493	17-Jun-98
		GB_BA1:U00011	40429	U00011	Mycobacterium leprae cosmid B1177.	Mycobacterium leprae	37,978	01-MAR-1994

TABLE 4: ALIGNMENT RESULTS

	GB_BA1:D90829	20277	D90829	E.coli genomic DNA, Kohara clone #337(41.9-42.3 min.).	Escherichia coli	35,750	21-MAR-1997
rxa00928 741	GB_PR2:HS1121J18	138145	AL031653	Human DNA sequence from clone 1121J18 on chromosome 20. Contains ESTs, STS, GSSs, a ca repeat polymorphism and genomic marker D20S115', complete sequence.	Homo sapiens	37,997	23-Nov-99
	GB_PR2:HS1121J18	138145	AL031653	Human DNA sequence from clone 1121J18 on chromosome 20. Contains ESTs, STS, GSSs, a ca repeat polymorphism and genomic marker D20S115', complete sequence.	Homo sapiens	38,701	23-Nov-99
	GB_HTG3:AC008715	101012	AC008715	Homo sapiens chromosome 5 clone CIT978SKB_84H3, *** SEQUENCING IN PROGRESS ***, 24 unordered pieces.	Homo sapiens	38,199	3-Aug-99
rxa00929 786	GB_HTG3:AC004480	220000	AC004480	Homo sapiens chromosome 4, *** SEQUENCING IN PROGRESS ***, 7 unordered pieces.	Homo sapiens	37,131	2-Sep-99
	GB_HTG3:AC004480	220000	AC004480	Homo sapiens chromosome 4, *** SEQUENCING IN PROGRESS ***, 7 unordered pieces.	Homo sapiens	37,131	2-Sep-99
	GB_HTG3:AC004480	220000	AC004480	Homo sapiens chromosome 4, *** SEQUENCING IN PROGRESS ***, 7 unordered pieces.	Homo sapiens	37,775	2-Sep-99
rxa00937 495	GB_GSS3:B67258	592	B67258	T23N5TF TAMU Arabidopsis thaliana genomic clone T23N5, genomic survey sequence.	Arabidopsis thaliana	35,644	09-DEC-1997
	GB_PL2:ATAC006413	96059	AC006413	Arabidopsis thaliana chromosome II BAC F5K7 genomic sequence, complete sequence.	Arabidopsis thaliana	36,864	09-MAR-1999
	GB_EST8:AA052151	282	AA052151	mf81g03.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:420724 5', mRNA sequence.	Mus musculus	38,652	13-Sep-96
rxa00938 381	GB_BA2:AF121000	19751	AF121000	Corynebacterium glutamicum strain 22243 R-plasmid pAG1, complete sequence.	Corynebacterium glutamicum	39,410	14-Apr-99
	GB_BA1:FVBPOAD2A	45519	D26094	Flavobacterium sp. plasmid pOAD2 DNA, whole sequence.	Flavobacterium sp.	37,228	6-Feb-99
	GB_BA1:FVBPOAD2A	45519	D26094	Flavobacterium sp. plasmid pOAD2 DNA, whole sequence.	Flavobacterium sp.	63,102	6-Feb-99
rxa00966 640	GB_BA1:MLCB628	40789	Y14967	Mycobacterium leprae cosmid B628.	Mycobacterium leprae	60,938	29-Aug-97
	GB_BA1:MLCB1770	37821	Z70722	Mycobacterium leprae cosmid B1770.	Mycobacterium leprae	60,938	29-Aug-97
	GB_BA1:MTCY21D4	20760	Z80775	Mycobacterium tuberculosis H37Rv complete genome; segment 3/262.	Mycobacterium tuberculosis	59,375	24-Jun-99
rxa00968 1054	GB_BA1:MSGY219	38721	AD000013	Mycobacterium tuberculosis sequence from clone y219.	Mycobacterium tuberculosis	36,077	10-DEC-1996
	GB_BA1:MTCY21D4	20760	Z80775	Mycobacterium tuberculosis H37Rv complete genome; segment 3/262.	Mycobacterium tuberculosis	67,536	24-Jun-99
	GB_BA1:MLCB628	40789	Y14967	Mycobacterium leprae cosmid B628.	Mycobacterium leprae	65,990	29-Aug-97
rxa00975 1773	GB_PAT:E14508	3579	E14508	DNA encoding Brevibacterium diaminopimelic acid decarboxylase and arginyl-tRNA synthase.	Corynebacterium glutamicum	99,887	28-Jul-99
	GB_PAT:AR038110	3579	AR038110	Sequence 15 from patent US 5804414.	Unknown.	99,887	29-Sep-99
	GB_PAT:E16355	3579	E16355	Brevibacterium argS and lysA genes.	Corynebacterium glutamicum	99,887	28-Jul-99
rxa00978 738	GB_PR2:HSAC000372	41730	AC000372	Human cosmid g1980a186, complete sequence.	Homo sapiens	34,674	12-MAR-1997
	GB_PR3:AC005503	40998	AC005503	Homo sapiens clone UWGC:g5129s003 from 7q31, complete sequence.	Homo sapiens	34,674	20-Aug-98
	GB_PR2:HSAC000372	41730	AC000372	Human cosmid g1980a186, complete sequence.	Homo sapiens	38,881	12-MAR-1997
rxa00985 2832	GB_BA1:MTV008	63033	AL021246	Mycobacterium tuberculosis H37Rv complete genome; segment 108/162.	Mycobacterium tuberculosis	38,126	17-Jun-98
	GB_BA1:BSVALTRS	3168	X77239	B.subtilis valS gene.	Bacillus subtilis	52,036	16-Apr-97
	GB_BA1:ECOUW93	338534	U14003	Escherichia coli K-12 chromosomal region from 92.8 to 00.1 minutes.	Escherichia coli	37,971	17-Apr-96

TABLE 4: ALIGNMENT RESULTS

rxa00998	585	GB_PAT:E13660	1916	E13660	gDNA encoding 6-phosphogluconate dehydrogenase.	Corynebacterium glutamicum	38,398	24-Jun-98
		GB_HTG2:AF164115	94757	AF164115	Homo sapiens chromosome 8 clone BAC 644F11, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	33,563	12-Jul-99
		GB_HTG2:AF164115	94757	AF164115	Homo sapiens chromosome 8 clone BAC 644F11, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	33,563	12-Jul-99
rxa01020	870	GB_EST29:AI553731	416	AI553731	tn28b06.x1 NCI_CGAP_Brn25 Homo sapiens cDNA clone IMAGE:2168915 3' similar to contains element TAR1 TAR1 repetitive element ;, mRNA sequence.	Homo sapiens	36,855	12-MAY-1999
		GB_EST35:AI871115	506	AI871115	wl79c08.x1 NCI_CGAP_Brn25 Homo sapiens cDNA clone IMAGE:2431118 3' similar to TR:O75176 O75176 KIAA0692 PROTEIN ;contains element MER15 repetitive element ;, mRNA sequence.	Homo sapiens	37,549	30-Aug-99
		GB_EST27:AI430328	520	AI430328	mf66a05.y1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:419216 5', mRNA sequence.	Mus musculus	37,765	09-MAR-1999
rxa01061	1061	GB_BA1:MTCY21D4	20760	Z80775	Mycobacterium tuberculosis H37Rv complete genome; segment 3/262.	Mycobacterium tuberculosis	62,606	24-Jun-99
		GB_BA1:MSGY219	38721	AD000013	Mycobacterium tuberculosis sequence from clone y219.	Mycobacterium tuberculosis	41,171	10-DEC-1996
		GB_BA1:MLCB628	40789	Y14967	Mycobacterium leprae cosmid B628.	Mycobacterium leprae	61,022	29-Aug-97
rxa01072	354	GB_BA2:AF112535	4363	AF112535	Corynebacterium glutamicum putative glutaredoxin NrdH (nrdH), NrdI (nrdI), and ribonucleotide reductase alpha-chain (nrdE) genes, complete cds.	Corynebacterium glutamicum	99,718	5-Aug-99
		GB_BA1:CANRDFGEN	6054	Y09572	Corynebacterium ammoniagenes nrdH, nrdI, nrdE, nrdF genes.	Corynebacterium ammoniagenes	62,393	18-Apr-98
		GB_BA1:MTCY22D7	31859	Z83866	Mycobacterium tuberculosis H37Rv complete genome; segment 133/162.	Mycobacterium tuberculosis	37,714	17-Jun-98
rxa01124	1602	GB_BA1:SC1C2	42210	AL031124	Streptomyces coelicolor cosmid 1C2.	Streptomyces coelicolor	60,616	15-Jan-99
		GB_BA1:MTV012	70287	AL021287	Mycobacterium tuberculosis H37Rv complete genome; segment 132/162.	Mycobacterium tuberculosis	37,913	23-Jun-99
		GB_BA1:MLCB637	44882	Z99263	Mycobacterium leprae cosmid B637.	Mycobacterium leprae	61,216	17-Sep-97
rxa01199	871	GB_PR3:AF046873	2153	AF046873	Homo sapiens synapsin IIIa mRNA, complete cds.	Homo sapiens	37,184	28-Apr-98
		GB_EST30:AI649049	691	AI649049	uk34f03.x1 Sugano mouse kidney mkia Mus musculus cDNA clone IMAGE:1970909 3' similar to gb:X15684 Mouse mRNA for liver-type glucose transporter protein (MOUSE);, mRNA sequence.	Mus musculus	37,226	30-Apr-99
		GB_EST23:AI121163	468	AI121163	ud70b04.x1 Sugano mouse liver mlia Mus musculus cDNA clone IMAGE:1451215 3' similar to gb:J03810 GLUCOSE TRANSPORTER TYPE 2, LIVER (HUMAN); gb:X15684 Mouse mRNA for liver-type glucose transporter protein (MOUSE);, mRNA sequence.	Mus musculus	35,057	2-Sep-98
rxa01223	735	GB_PR4:AC007386	176742	AC007386	Homo sapiens BAC clone NH0359K10 from 2, complete sequence.	Homo sapiens	39,551	22-OCT-1999
		GB_PR4:AC007386	176742	AC007386	Homo sapiens BAC clone NH0359K10 from 2, complete sequence.	Homo sapiens	38,678	22-OCT-1999
rxa01226	663	GB_PR2:HS21F7	150789	AL033375	Human DNA sequence from clone 21F7 on chromosome 6q16.1-21.Contains part of an exon of a putative new gene and STSs and GSSs, complete sequence.	Homo sapiens	37,309	23-Nov-99
		GB_PR3:AF023268	75270	AF023268	Homo sapiens clk2 kinase (CLK2), propin1, cote1, glucocerebrosidase (GBA), and metaxin genes, complete cds; metaxin pseudogene and glucocerebrosidase pseudogene; and thrombospondin3 (THBS3) gene, partial cds.	Homo sapiens	38,923	28-OCT-1997
		GB_BA2:AF016485	191346	AF016485	Halobacterium sp. NRC-1 plasmid pNRC100, complete plasmid sequence.	Halobacterium sp. NRC-1	39,938	29-MAR-1999

TABLE 4: ALIGNMENT RESULTS

rxa01228 339	GB_PR2:HS1158E12	163871	AL031584	Human DNA sequence from clone 1158E12 on chromosome Xp11.23-11.4	Homo sapiens	34,718	23-Nov-99
	GB_HTG6:AC008180_0	110000	AC008180	Contains EST, STS, GSS, CpG island, complete sequence. Homo sapiens clone RP11-292L5, *** SEQUENCING IN PROGRESS ***, 152 unordered pieces.	Homo sapiens	31,212	29-Jul-99
rxa01252 777	GB_PR4:AC004908	138251	AC004908	Homo sapiens PAC clone DJ0855D21, complete sequence.	Homo sapiens	37,082	15-Jan-99
	GB_BA1:MTV025	121125	AL022121	Mycobacterium tuberculosis H37Rv complete genome; segment 155/162.	Mycobacterium tuberculosis	39,171	24-Jun-99
	GB_BA1:SC66T3	35101	AL079348	Streptomyces coelicolor cosmid 66T3.	Streptomyces coelicolor	35,401	19-Jun-99
	GB_BA2:AF151381	1296	AF151381	Streptomyces coelicolor recombination protein RecR (recR) gene, complete cds; and unknown gene.	Streptomyces coelicolor	53,826	20-Aug-99
rxa01264 339	GB_GSS10:AQ195163	617	AQ195163	RPCI11-66I23.TJ RPCI-11 Homo sapiens genomic clone RPCI-11-66I23, genomic survey sequence.	Homo sapiens	41,016	20-Apr-99
	GB_HTG2:AC000016	194000	AC000016	Homo sapiens chromosome 4, *** SEQUENCING IN PROGRESS ***, 9 unordered pieces.	Homo sapiens	38,253	16-MAY-1998
	GB_STS:G53604	617	G53604	SHGC-86312 Human Homo sapiens STS genomic, sequence tagged site.	Homo sapiens	41,016	25-Jun-99
rxa01265							
rxa01274 1218	GB_PAT:E15823	2323	E15823	DNA encoding cell surface protein from Corynebacterium ammoniagenes.	Corynebacterium ammoniagenes	52,523	28-Jul-99
	GB_HTG3:AF182108	167065	AF182108	Homo sapiens chromosome 8 clone BAC R-11N9 map 8p12. 8, ***SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	35,377	08-OCT-1999
	GB_HTG3:AF182108	167065	AF182108	Homo sapiens chromosome 8 clone BAC R-11N9 map 8p12. 8, ***SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	35,377	08-OCT-1999
rxa01278 2250	GB_BA1:MLB1790G	37617	Z14314	M.leprae genes rplL, rpoB, rpoC, end, rpsL, rpsG, efg, tuf, rpsJ, rplC for ribosomal protein L7, RNA polymerase beta subunit, RNA polymerase beta' subunit, endonuclease, ribosomal protein S7, ribosomal protein S12, elongation factor G, elongation factor Tu, ribosomal protein S10, ribosomal protein L3 and mkl gene.	Mycobacterium leprae	70,031	11-Feb-93
rxa01283 1316	GB_BA1:MTV040	15100	AL021943	Mycobacterium tuberculosis H37Rv complete genome; segment 33/162.	Mycobacterium tuberculosis	70,704	17-Jun-98
	GB_BA1:ATFUSATUF	3412	X99673	A.tumefaciens fusA & tufA genes.	Agrobacterium tumefaciens	64,042	11-Nov-96
	GB_BA1:MLB1790G	37617	Z14314	M.leprae genes rplL, rpoB, rpoC, end, rpsL, rpsG, efg, tuf, rpsJ, rplC for ribosomal protein L7, RNA polymerase beta subunit, RNA polymerase beta' subunit, endonuclease, ribosomal protein S7, ribosomal protein S12, elongation factor G, elongation factor Tu, ribosomal protein S10, ribosomal protein L3 and mkl gene.	Mycobacterium leprae	65,865	11-Feb-93
	GB_BA1:MTCI376	19770	Z95972	Mycobacterium tuberculosis H37Rv complete genome; segment 32/162.	Mycobacterium tuberculosis	64,633	17-Jun-98
	GB_BA2:ECOUW89	176195	U00006	E. coli chromosomal region from 89.2 to 92.8 minutes.	Escherichia coli	46,615	17-DEC-1993
rxa01284 667	GB_BA1:CGTUF	1191	X77034	C.glutamicum tuf gene for elongation factor Tu.	Corynebacterium glutamicum	100,000	27-OCT-1994
	GB_BA1:MTCY210	36804	Z84395	Mycobacterium tuberculosis H37Rv complete genome; segment 34/162.	Mycobacterium tuberculosis	74,622	17-Jun-98
	GB_BA1:MSGY42	36526	AD000005	Mycobacterium tuberculosis sequence from clone y42.	Mycobacterium tuberculosis	37,419	03-DEC-1996

09602839 . 062300

TABLE 4: ALIGNMENT RESULTS

rxa01327	267	GB_SY:SCU53587	4546	U53587	Artificial Corynebacterium glutamicum IS1207-derived transposon transposase genes, complete cds, and 3'5"-aminoglycoside phosphotransferase (aphA-3) gene, complete cds.	synthetic construct	60,674	06-MAY-1996
		GB_BA1:BLIS13869	1840	Z66534	B.lactofermentum IS13869 DNA and transposase gene.	Corynebacterium glutamicum	62,172	16-Jul-96
		EM_PAT:E10419	1469	E10419	Insertion sequence derived from C.glutamicum.	Corynebacterium glutamicum	60,674	08-OCT-1997 (Rel. 52, Created)
rxa01328	498	GB_BA1:BLIS13869	1840	Z66534	B.lactofermentum IS13869 DNA and transposase gene.	Corynebacterium glutamicum	73,038	16-Jul-96
		GB_SY:SCU53587	4546	U53587	Artificial Corynebacterium glutamicum IS1207-derived transposon transposase genes, complete cds, and 3'5"-aminoglycoside phosphotransferase (aphA-3) gene, complete cds.	synthetic construct	68,813	06-MAY-1996
		GB_PAT:I43826	1452	I43826	Sequence 1 from patent US 5633154.	Unknown.	69,014	07-OCT-1997
rxa01329	414	GB_BA1:BLIS13869	1840	Z66534	B.lactofermentum IS13869 DNA and transposase gene.	Corynebacterium glutamicum	73,966	16-Jul-96
		GB_PAT:E12758	1453	E12758	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	73,020	24-Jun-98
		GB_PAT:I33166	1453	I33166	Sequence 1 from patent US 5591577.	Unknown.	73,020	6-Feb-97
rxa01344	2647	GB_BA1:MSU24494	3752	U24494	Mycobacterium smegmatis DNA polymerase (rpoB) gene, complete cds.	Mycobacterium smegmatis	73,086	07-MAR-1996
		GB_BA1:MTCI376	19770	Z95972	Mycobacterium tuberculosis H37Rv complete genome; segment 32/162.	Mycobacterium tuberculosis	71,385	17-Jun-98
		GB_BA1:MSGRPOB	5084	L27989	Mycobacterium tuberculosis RNA polymerase beta-subunit (rpoB) gene, complete cds and RNA polymerase beta'-subunit rpoC gene, partial cds.	Mycobacterium tuberculosis	71,429	13-Sep-94
rxa01355	909	GB_HTG4:AC009135	168607	AC009135	Homo sapiens chromosome 16 clone RPCI-11_509E10, *** SEQUENCING IN PROGRESS ***, 231 unordered pieces.	Homo sapiens	37,156	31-OCT-1999
		GB_HTG4:AC009135	168607	AC009135	Homo sapiens chromosome 16 clone RPCI-11_509E10, *** SEQUENCING IN PROGRESS ***, 231 unordered pieces.	Homo sapiens	37,156	31-OCT-1999
		GB_BA1:PFLEPALEP	1391	X56466	P.fluorescens lepA (partial) and lep gene for leader peptidase 1.	Pseudomonas fluorescens	44,023	5-Feb-92
rxa01387	469	GB_BA1:MLB1790G	37617	Z14314	M.leprae genes rplL, rpoB, rpoC, end, rpsL, rpsG, efg, tuf, rpsJ, rplC for ribosomal protein L7, RNA polymerase beta subunit, RNA polymerase beta' subunit, endonuclease, ribosomal protein S7, ribosomal protein S12, elongation factor G, elongation factor Tu, ribosomal protein S10, ribosomal protein L3 and mkl gene.	Mycobacterium leprae	71,429	11-Feb-93
		GB_BA1:MTCI376	19770	Z95972	Mycobacterium tuberculosis H37Rv complete genome; segment 32/162.	Mycobacterium tuberculosis	73,176	17-Jun-98
		GB_BA1:BSUB0001	213080	Z99104	Bacillus subtilis complete genome (section 1 of 21): from 1 to 213080.	Bacillus subtilis	63,853	26-Nov-97
rxa01388	255	GB_HTG2:HS676J13	117045	AL034347	Homo sapiens chromosome 6 clone RP4-676J13 map q14, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	36,863	03-DEC-1999
		GB_HTG2:HS676J13	117045	AL034347	Homo sapiens chromosome 6 clone RP4-676J13 map q14, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	36,863	03-DEC-1999
		GB_HTG2:HS676J13	117045	AL034347	Homo sapiens chromosome 6 clone RP4-676J13 map q14, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	29,804	03-DEC-1999
		GB_BA1:MTV012	70287	AL021287	Mycobacterium tuberculosis H37Rv complete genome; segment 132/162.	Mycobacterium tuberculosis	36,547	23-Jun-99
rxa01398	659	GB_BA1:S70345	5077	S70345	SpaA=endocarditis immunodominant antigen [Streptococcus sobrinus, MUCOB 263, Genomic, 5077 nt].	Streptococcus sobrinus	35,139	23-Sep-94
		GB_BA1:STRPAGA	5100	D90354	S.sobrinus pag gene for surface protein antigen (PAg).	Streptococcus sobrinus	35,604	7-Feb-99

TABLE 4: ALIGNMENT RESULTS

rxa01431	444	GB_BA2:AE001648	13965	AE001648	Chlamydia pneumoniae section 64 of 103 of the complete genome.	Chlamydophila pneumoniae	44,218	08-MAR-1999
		GB_BA2:AE001648	13965	AE001648	Chlamydia pneumoniae section 64 of 103 of the complete genome.	Chlamydophila pneumoniae	35,520	08-MAR-1999
rxa01432	1074	GB_BA1:MSGY367	35336	AD000008	Mycobacterium tuberculosis sequence from clone y367.	Mycobacterium tuberculosis	37,869	03-DEC-1996
		GB_BA1:MTV028	11381	AL021426	Mycobacterium tuberculosis H37Rv complete genome; segment 162/162.	Mycobacterium tuberculosis	61,891	17-Jun-98
		GB_BA2:AF023161	1775	AF023161	Mycobacterium smegmatis thioredoxin reductase (trxB) and thioredoxin (trxA) genes, complete cds.	Mycobacterium smegmatis	64,105	13-OCT-1997
rxa01433	726	GB_BA2:AF105341	3010	AF105341	Listeria monocytogenes threonine dehydratase (thd1) gene, partial cds; alpha acetolactate decarboxylase gene, complete cds; and pyrimidine nucleoside phosphorylase (pdp1) gene, partial cds.	Listeria monocytogenes	36,254	04-MAR-1999
		GB_BA2:AF105341	3010	AF105341	Listeria monocytogenes threonine dehydratase (thd1) gene, partial cds; alpha acetolactate decarboxylase gene, complete cds; and pyrimidine nucleoside phosphorylase (pdp1) gene, partial cds.	Listeria monocytogenes	35,303	04-MAR-1999
rxa01443	954	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	72,823	9-Aug-95
		GB_PAT:I33168	1279	I33168	Sequence 4 from patent US 5591577.	Unknown.	72,293	6-Feb-97
		GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	72,293	24-Jun-98
rxa01444	390	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	69,034	9-Aug-95
		GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	69,318	24-Jun-98
		GB_PAT:I33168	1279	I33168	Sequence 4 from patent US 5591577.	Unknown.	69,318	6-Feb-97
rxa01449	1141	GB_HTG1:CEY1A5	196643	AL008872	Caenorhabditis elegans chromosome III clone Y1A5, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	36,208	9-Nov-97
		GB_HTG1:CEY1A5	196643	AL008872	Caenorhabditis elegans chromosome III clone Y1A5, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	36,208	9-Nov-97
		GB_IN1:PFMAL3P4	113899	AL008970	Plasmodium falciparum MAL3P4, complete sequence.	Plasmodium falciparum	33,333	28-Jul-99
rxa01490	1014	GB_BA1:MTV002	56414	AL008967	Mycobacterium tuberculosis H37Rv complete genome; segment 122/162.	Mycobacterium tuberculosis	36,436	17-Jun-98
		GB_BA1:SC9F2	11908	AL035559	Streptomyces coelicolor cosmid 9F2.	Streptomyces coelicolor	36,774	25-Feb-99
		GB_BA1:SPSNBCGEN	22449	X98690	S.pristinaespiralis snbC and snbDE genes.	Streptomyces pristinaespiralis	41,509	24-MAR-1997
rxa01493	1434	GB_HTG3:AC009583	172341	AC009583	Homo sapiens chromosome 4 clone 158_C_21 map 4, *** SEQUENCING IN PROGRESS ***, 17 unordered pieces.	Homo sapiens	34,102	29-Sep-99
		GB_HTG3:AC009583	172341	AC009583	Homo sapiens chromosome 4 clone 158_C_21 map 4, *** SEQUENCING IN PROGRESS ***, 17 unordered pieces.	Homo sapiens	34,102	29-Sep-99
		GB_HTG3:AC009583	172341	AC009583	Homo sapiens chromosome 4 clone 158_C_21 map 4, *** SEQUENCING IN PROGRESS ***, 17 unordered pieces.	Homo sapiens	35,133	29-Sep-99
rxa01496	3135	GB_BA1:MTCY16B7	43430	Z81331	Mycobacterium tuberculosis H37Rv complete genome; segment 123/162.	Mycobacterium tuberculosis	39,391	17-Jun-98
		GB_BA1:MSGY414A	40121	AD000007	Mycobacterium tuberculosis sequence from clone y414a.	Mycobacterium tuberculosis	60,308	03-DEC-1996
		GB_BA1:MLCB596	38426	AL035472	Mycobacterium leprae cosmid B596.	Mycobacterium leprae	57,989	27-Aug-99
rxa01522	1701	GB_BA2:RHMGLTX	4119	M27221	Sinorhizobium meliloti glutamyl-tRNA synthetase (gltX) and lysyl-tRNA synthetase (lysS) genes, complete cds.	Sinorhizobium meliloti	49,669	11-Sep-98
		GB_BA1:MTCY06H11	38000	Z85982	Mycobacterium tuberculosis H37Rv complete genome; segment 73/162.	Mycobacterium tuberculosis	38,152	17-Jun-98

TABLE 4: ALIGNMENT RESULTS

	GB_EST8:AA002902	396	AA002902	mg38a12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:426046 5', mRNA sequence.	Mus musculus	42,333	19-Jul-96
rxa01556 872	GB_PR2:HSU73633	42845	U73633	Human chromosome 11 146h12 cosmid, complete sequence.	Homo sapiens	37,412	19-Jun-97
	GB_RO:MMU70209	14141	U70209	Mus musculus polycystic kidney disease 1 protein (Pkd1) mRNA, complete cds.	Mus musculus	42,536	31-MAY-1997
	GB_HTG2:AC007903	170591	AC007903	Homo sapiens chromosome 18 clone 563_I_8 map 18, *** SEQUENCING IN PROGRESS ***, 6 unordered pieces.	Homo sapiens	34,868	23-Jun-99
rxa01558 1332	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	38,567	17-Jun-98
	GB_BA1:MLCB1259	38807	AL023591	Mycobacterium leprae cosmid B1259.	Mycobacterium leprae	53,364	27-Aug-99
	GB_BA1:U00011	40429	U00011	Mycobacterium leprae cosmid B1177.	Mycobacterium leprae	38,498	01-MAR-1994
rxa01559 1965	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	37,945	17-Jun-98
	GB_BA1:MLCB1259	38807	AL023591	Mycobacterium leprae cosmid B1259.	Mycobacterium leprae	51,117	27-Aug-99
	GB_BA1:U00011	40429	U00011	Mycobacterium leprae cosmid B1177.	Mycobacterium leprae	37,513	01-MAR-1994
rxa01582 1212	GB_BA1:MTCY06H11	38000	Z85982	Mycobacterium tuberculosis H37Rv complete genome; segment 73/162.	Mycobacterium tuberculosis	60,249	17-Jun-98
	GB_BA1:MSGB1133CS	42106	L78811	Mycobacterium leprae cosmid B1133 DNA sequence.	Mycobacterium leprae	58,547	15-Jun-96
	GB_BA1:SCI35	40909	AL031541	Streptomyces coelicolor cosmid I35.	Streptomyces coelicolor	37,479	9-Sep-98
rxa01583 2466	GB_BA1:MTV004	69350	AL009198	Mycobacterium tuberculosis H37Rv complete genome; segment 144/162.	Mycobacterium tuberculosis	39,373	18-Jun-98
	GB_GSS8:AQ077749	538	AQ077749	CIT-HSP-2367E24.TR CIT-HSP Homo sapiens genomic clone 2367E24, genomic survey sequence.	Homo sapiens	36,989	20-Aug-98
	GB_BA1:MTV004	69350	AL009198	Mycobacterium tuberculosis H37Rv complete genome; segment 144/162.	Mycobacterium tuberculosis	39,220	18-Jun-98
rxa01596 1902	GB_BA1:SCI51	40745	AL109848	Streptomyces coelicolor cosmid I51.	Streptomyces coelicolor A3(2)	38,388	16-Aug-99
	GB_BA1:MTCI125	37432	Z98268	Mycobacterium tuberculosis H37Rv complete genome; segment 76/162.	Mycobacterium tuberculosis	53,052	17-Jun-98
	GB_BA1:MTHYPROT	2544	X98295	M.tuberculosis TlyA gene.	Mycobacterium tuberculosis	49,393	2-Jun-98
rxa01601 1035	GB_BA1:MTCI125	37432	Z98268	Mycobacterium tuberculosis H37Rv complete genome; segment 76/162.	Mycobacterium tuberculosis	54,801	17-Jun-98
	GB_BA1:MLCB1351	38936	Z95117	Mycobacterium leprae cosmid B1351.	Mycobacterium leprae	39,577	24-Jun-97
	GB_BA1:U00021	39193	U00021	Mycobacterium leprae cosmid L247.	Mycobacterium leprae	39,476	29-Sep-94
rxa01613 1338	GB_BA1:MTCY24A1	20270	Z95207	Mycobacterium tuberculosis H37Rv complete genome; segment 124/162.	Mycobacterium tuberculosis	52,216	17-Jun-98
	GB_BA1:AF002193	1812	AF002193	Mycobacterium tuberculosis glutathione reductase homolog (gorA) gene, complete cds.	Mycobacterium tuberculosis	52,216	18-Jul-97
	GB_HTG3:AC008675	206439	AC008675	Homo sapiens chromosome 5 clone CIT978SKB_45I8, *** SEQUENCING IN PROGRESS ***, 43 unordered pieces.	Homo sapiens	36,145	3-Aug-99
rxa01621 1563	GB_BA1:MTY15F10	38204	Z94121	Mycobacterium tuberculosis H37Rv complete genome; segment 161/162.	Mycobacterium tuberculosis	36,776	17-Jun-98
	GB_BA1:MSGY367	35336	AD000008	Mycobacterium tuberculosis sequence from clone y367.	Mycobacterium tuberculosis	60,525	03-DEC-1996
	GB_BA1:MTY15F10	38204	Z94121	Mycobacterium tuberculosis H37Rv complete genome; segment 161/162.	Mycobacterium tuberculosis	36,288	17-Jun-98
rxa01648 492	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	76,483	9-Aug-95
	GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	75,574	29-Sep-99
	GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	75,574	24-Jun-98
rxa01649 543	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	67,978	9-Aug-95
	GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	67,857	29-Sep-99
	GB_PAT:E12760	1279	E12760	DNA encoding Brevibacterium transposase.	Corynebacterium glutamicum	67,857	24-Jun-98
rxa01650 237	GB_PL2:SPAC17A2	36642	Z99292	S.pombe chromosome I cosmid c17A2.	Schizosaccharomyces pombe	42,241	22-Jul-99
	GB_PL2:SPAC17A2	36642	Z99292	S.pombe chromosome I cosmid c17A2.	Schizosaccharomyces pombe	33,766	22-Jul-99

TABLE 4: ALIGNMENT RESULTS

	GB_PL1:SCYDR012W	2732	Z74308	S.cerevisiae chromosome IV reading frame ORF YDR012w.	Saccharomyces cerevisiae	30,804	12-Aug-98
rxa01651 258	GB_BA1:CGISABL	1290	X69104	C.glutamicum IS3 related insertion element.	Corynebacterium glutamicum	69,643	9-Aug-95
	GB_PAT:AR038104	1279	AR038104	Sequence 9 from patent US 5804414.	Unknown.	67,265	29-Sep-99
	GB_PAT:I33168	1279	I33168	Sequence 4 from patent US 5591577.	Unknown.	67,265	6-Feb-97
rxa01670 930	GB_BA2:SMU56906	3303	U56906	Serratia marcescens DNA gyrase (gyrA) gene, complete cds.	Serratia marcescens	36,186	7-Jan-98
	GB_BA1:D90902	122056	D90902	Synechocystis sp. PCC6803 complete genome, 4/27, 402290-524345.	Synechocystis sp.	37,814	7-Feb-99
	GB_HTG2:HSDJ816K9	144277	AL117349	Homo sapiens chromosome 1 clone RP4-816K9, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Homo sapiens	41,759	30-Nov-99
rxa01680							
rxa01704 1100	GB_HTG2:AF129075	195012	AF129075	Homo sapiens chromosome 21 clone J12100; E0479 map 21q22.1, ***SEQUENCING IN PROGRESS ***, in ordered pieces.	Homo sapiens	40,187	03-MAR-1999
	GB_HTG2:AF129075	195012	AF129075	Homo sapiens chromosome 21 clone J12100; E0479 map 21q22.1, ***SEQUENCING IN PROGRESS ***, in ordered pieces.	Homo sapiens	40,187	03-MAR-1999
	GB_HTG2:AC007271	184269	AC007271	Homo sapiens clone NH0004B12, *** SEQUENCING IN PROGRESS ***, 2 unordered pieces.	Homo sapiens	38,667	16-Apr-99
rxa01710 531	GB_BA1:MTCY441	35187	Z80225	Mycobacterium tuberculosis H37Rv complete genome; segment 118/162.	Mycobacterium tuberculosis	56,309	18-Jun-98
	GB_EST16:AA540562	695	AA540562	LD20282.5prime LD Drosophila melanogaster embryo BlueScript Drosophila melanogaster cDNA clone LD20282 5prime, mRNA sequence.	Drosophila melanogaster	51,357	28-Nov-98
	GB_EST37:AI944677	580	AI944677	bs04b04.y1 Drosophila melanogaster adult testis library Drosophila melanogaster cDNA clone bs04b04 5', mRNA sequence.	Drosophila melanogaster	50,728	17-Aug-99
rxa01724 1343	GB_BA1:MLU15186	36241	U15186	Mycobacterium leprae cosmid L471.	Mycobacterium leprae	37,412	09-MAR-1995
	GB_BA1:MTCY373	35516	Z73419	Mycobacterium tuberculosis H37Rv complete genome; segment 57/162.	Mycobacterium tuberculosis	47,819	17-Jun-98
	GB_HTG2:AC007608	170057	AC007608	Homo sapiens chromosome 16 clone 401P9, *** SEQUENCING IN PROGRESS***, 59 unordered pieces.	Homo sapiens	37,236	20-MAY-1999
rxa01725 330	GB_BA1:MTCY373	35516	Z73419	Mycobacterium tuberculosis H37Rv complete genome; segment 57/162.	Mycobacterium tuberculosis	75,610	17-Jun-98
	GB_BA1:MLU15186	36241	U15186	Mycobacterium leprae cosmid L471.	Mycobacterium leprae	39,355	09-MAR-1995
	GB_BA1:PSERHO	1479	L27278	Pseudomonas fluorescens rho gene, complete cds.	Pseudomonas fluorescens	63,303	9-Jan-95
rxa01726 696	GB_BA1:MTCY373	35516	Z73419	Mycobacterium tuberculosis H37Rv complete genome; segment 57/162.	Mycobacterium tuberculosis	72,899	17-Jun-98
	GB_BA1:MLU15186	36241	U15186	Mycobacterium leprae cosmid L471.	Mycobacterium leprae	37,500	09-MAR-1995
	GB_BA1:SLRHOGENE	2986	X95444	S.lividans Rho gene.	Streptomyces lividans	69,065	1-Feb-96
rxa01730 1804	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	39,943	17-Jun-98
	GB_BA1:MLCB1259	38807	AL023591	Mycobacterium leprae cosmid B1259.	Mycobacterium leprae	65,120	27-Aug-99
	GB_BA2:S82268	2209	S82268	Mycobacterium leprae ASPS and antigen T5 genes, complete cds.	Mycobacterium leprae	40,715	22-Jul-98
rxa01733 1274	GB_BA1:MSORIREP	10430	X92503	M.smegmatis origin of replication and genes rpmH, dnaA, dnaN, gnd, recF, gyrB, gyrA.	Mycobacterium smegmatis	52,740	26-Aug-97
	GB_BA1:MSGYRBA	6000	X94224	M.smegmatis gyrB and gyrA genes.	Mycobacterium smegmatis	52,277	12-Feb-97
	GB_HTG4:AC010890	175554	AC010890	Homo sapiens chromosome unknown clone NH0449L24, WORKING DRAFT SEQUENCE, in unordered pieces.	Homo sapiens	36,601	29-OCT-1999
rxa01736 2891	GB_BA1:MTV014	58280	AL021646	Mycobacterium tuberculosis H37Rv complete genome; segment 137/162.	Mycobacterium tuberculosis	38,918	18-Jun-98
	GB_PL2:AF156928	2290	AF156928	Candida albicans folylpolyglutamate synthetase (fpgs) gene, complete cds.	Candida albicans	34,894	22-Jun-99

09602839 . 062300

TABLE 4: ALIGNMENT RESULTS

	GB_GSS12:AQ421204	483	AQ421204	RPCI-11-167B4.TJ RPCI-11 Homo sapiens genomic clone RPCI-11-167B4, genomic survey sequence.	Homo sapiens	39,085	23-MAR-1999
rxa01737 1182	GB_BA1:SCGD3	33779	AL096822	Streptomyces coelicolor cosmid GD3.	Streptomyces coelicolor	38,054	8-Jul-99
	GB_HTG1:CNS01DSB	222193	AL121768	Homo sapiens chromosome 14 clone R-976B16, *** SEQUENCING IN PROGRESS ***, in ordered pieces.	Homo sapiens	35,147	05-OCT-1999
	GB_HTG1:CNS01DSB	222193	AL121768	Homo sapiens chromosome 14 clone R-976B16, *** SEQUENCING IN PROGRESS ***, in ordered pieces.	Homo sapiens	35,147	05-OCT-1999
rxa01784 705	GB_BA2:AF121000	19751	AF121000	Corynebacterium glutamicum strain 22243 R-plasmid pAG1, complete sequence.	Corynebacterium glutamicum	36,270	14-Apr-99
	GB_BA1:FVBPOAD2A	45519	D26094	Flavobacterium sp. plasmid pOAD2 DNA, whole sequence.	Flavobacterium sp.	38,450	6-Feb-99
	GB_BA1:FVBPOAD2A	45519	D26094	Flavobacterium sp. plasmid pOAD2 DNA, whole sequence.	Flavobacterium sp.	59,052	6-Feb-99
	GB_IN1:AB018440	13738	AB018440	Echinococcus multilocularis mitochondrial DNA, complete genome.	Mitochondrion Echinococcus	34,877	28-OCT-
rxa01798 373	GB_BA1:SSU82227	8313	U82227	Sulfolobus solfataricus leucyl-tRNA synthetase (leuS) gene, partial cds, histidine biosynthesis operon hisCGABdFDEHI, (hisC, hisG,hisBd, hisF, hisD, hisE, hisH and hisI) genes, complete cds and seryl-tRNA synthetase (serS) gene, partial cds.	Sulfolobus solfataricus	40,166	14-Jul-97
	GB_BA1:SSU82227	8313	U82227	Sulfolobus solfataricus leucyl-tRNA synthetase (leuS) gene, partial cds, histidine biosynthesis operon hisCGABdFDEHI, (hisC, hisG, hisBd, hisF, hisD, hisE, hisH and hisI) genes, complete cds and seryl-tRNA synthetase (serS) gene, partial cds.	Sulfolobus solfataricus	33,989	14-Jul-97
rxa01818 1110	GB_IN1:CEF08G5	32784	Z70682	Caenorhabditis elegans cosmid F08G5, complete sequence.	Caenorhabditis elegans	35,032	23-Jul-99
	GB_HTG2:AC008029	123186	AC008029	Drosophila melanogaster chromosome 3 clone BACR01C11 (D819) RPCI-98 01.C.11 map 84D-84D strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 92 unordered pieces.	Drosophila melanogaster	35,197	2-Aug-99
	GB_HTG2:AC008029	123186	AC008029	Drosophila melanogaster chromosome 3 clone BACR01C11 (D819) RPCI-98 01.C.11 map 84D-84D strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 92 unordered pieces.	Drosophila melanogaster	35,197	2-Aug-99
rxa01819 570	GB_BA1:AB023076	4953	AB023076	Pseudomonas syringae DNA, the left outside of the hrpL homology region, strain:KW11.	Pseudomonas syringae	36,852	26-Feb-99
	GB_BA1:AB023076	4953	AB023076	Pseudomonas syringae DNA, the left outside of the hrpL homology region, strain:KW11.	Pseudomonas syringae	39,646	26-Feb-99
rxa01837 900	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	53,182	17-Jun-98
	GB_HTG2:AC006779	119562	AC006779	Caenorhabditis elegans clone Y47D7, *** SEQUENCING IN PROGRESS ***, 32 unordered pieces.	Caenorhabditis elegans	34,783	25-Feb-99
	GB_HTG2:AC006779	119562	AC006779	Caenorhabditis elegans clone Y47D7, *** SEQUENCING IN PROGRESS ***, 32 unordered pieces.	Caenorhabditis elegans	34,783	25-Feb-99
rxa01841 486	GB_BA2:AF139249	1383	AF139249	Actinobacillus actinomycetemcomitans rough colony protein A (rcpA) gene, complete cds.	Actinobacillus actinomycetemcomitans	37,395	25-MAY-1999
	GB_EST17:C76899	603	C76899	C76899 Mouse 3.5-dpc blastocyst cDNA Mus musculus cDNA clone J0022E02 3' similar to M.musculus DNA for LINE-1 or L1 element, mRNA sequence.	Mus musculus	44,828	25-Jun-98
	GB_PR3:U94190	6469	U94190	Homo sapiens Duo mRNA, complete cds.	Homo sapiens	38,382	04-MAY-1998
rxa01852 1410	GB_BA1:MTCY227	35946	Z77724	Mycobacterium tuberculosis H37Rv complete genome; segment 114/162.	Mycobacterium tuberculosis	38,378	17-Jun-98
	GB_BA1:MLCB1259	38807	AL023591	Mycobacterium leprae cosmid B1259.	Mycobacterium leprae	59,574	27-Aug-99

TABLE 4: ALIGNMENT RESULTS

	GB_BA1:U00011	40429	U00011	Mycobacterium leprae cosmid B1177.	Mycobacterium leprae	37,690	01-MAR-1994
					Rhizobium leguminosarum	39,401	23-Sep-92
rxa01862 1329	GB_BA1:RLDCTA	5820	Z11529	R.leguminosarum dctA gene encoding C4-dicarboxylate permease.	Rhizobium leguminosarum	39,401	12-Sep-93
	GB_BA1:RLDCTBD	3360	X06253	Rhizobium leguminosarum dctB and dctD genes involved in C4-dicarboxylate transport.			
	GB_BA1:RLDCTA	5820	Z11529	R.leguminosarum dctA gene encoding C4-dicarboxylate permease.	Rhizobium leguminosarum	39,269	23-Sep-92
					Bacillus subtilis	35,673	26-Nov-97
rxa01863 1219	GB_BA1:BSUB0005	208430	Z99108	Bacillus subtilis complete genome (section 5 of 21): from 802821 to 1011250.	Bacillus subtilis	57,261	20-Nov-97
	GB_BA1:D83967	22197	D83967	Bacillus subtilis genomic DNA, 74 degree region.	Staphylococcus aureus	99,595	26-Apr-93
	GB_BA1:STAATTB	300	M20393	S.aureus bacteriophage phi-11 attachment site (attB).	Trypanosoma brucei	42,034	22-Jun-99
rxa01872 928	GB_GSS15:AQ651661	422	AQ651661	Sheared DNA-5N18.TR Sheared DNA Trypanosoma brucei genomic clone Sheared DNA-5N18, genomic survey sequence.			
	GB_GSS15:AQ639444	175	AQ639444	927P1-17G6.TV 927P1 Trypanosoma brucei genomic clone 927P1-17G6, genomic survey sequence.	Trypanosoma brucei	51,786	8-Jul-99
	GB_HTG3:AC009919	134724	AC009919	Homo sapiens clone 115_I_23, LOW-PASS SEQUENCE SAMPLING.	Homo sapiens	37,222	8-Sep-99
rxa01878 1002	GB_HTG1:CEY64F11	177748	Z99776	Caenorhabditis elegans chromosome IV clone Y64F11, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	37,564	14-OCT-1998
	GB_HTG1:CEY64F11	177748	Z99776	Caenorhabditis elegans chromosome IV clone Y64F11, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	37,564	14-OCT-1998
	GB_HTG1:CEY64F11	177748	Z99776	Caenorhabditis elegans chromosome IV clone Y64F11, *** SEQUENCING IN PROGRESS ***, in unordered pieces.	Caenorhabditis elegans	37,576	14-OCT-1998
					Mycobacterium tuberculosis	39,631	19-Jun-98
rxa01913 948	GB_BA1:MTCY274	39991	Z74024	Mycobacterium tuberculosis H37Rv complete genome; segment 126/162.	Streptomyces coelicolor	58,226	4-Jun-98
	GB_BA1:SC2E1	38962	AL023797	Streptomyces coelicolor cosmid 2E1.	Streptomyces ramocissimus	58,009	15-OCT-1999
	GB_BA2:AF130345	965	AF130345	Streptomyces ramocissimus elongation factor Ts (tsf) gene, complete cds.			
					Mycobacterium tuberculosis	38,976	17-Jun-98
rxa01938 1551	GB_BA1:MTCY24A1	20270	Z95207	Mycobacterium tuberculosis H37Rv complete genome; segment 124/162.	Arabidopsis thaliana	54,028	28-Jun-99
	GB_GSS1:CNS00WZY	720	AL094252	Arabidopsis thaliana genome survey sequence SP6 end of BAC T12O8 of TAMU library from strain Columbia of Arabidopsis thaliana, genomic survey sequence.			
	GB_PR2:AP000056	100000	AP000056	Homo sapiens genomic DNA, chromosome 21q22.1, segment 27/28, complete sequence.	Homo sapiens	36,967	20-Nov-99
rxa01953 504	GB_BA1:MSGTNP	2276	M76495	Mycobacterium smegmatis insertion element tnpR and tnpA genes, complete cds.	Mycobacterium smegmatis	38,153	26-Apr-96
	GB_BA2:E12PHEAB	6164	M57500	Plasmid pEST1226 putative transposase (tnpA), catechol 1,2-dioxygenase (pheB), phenol monooxygenase (pheA), and putative transposase (tnpA) genes, complete cds.	Plasmid pEST1226	56,338	21-OCT-1998
	GB_PR2:HS179N16	172048	Z95152	Homo sapiens DNA sequence from PAC 179N16 on chromosome 6p21.1-21.33. Contains the SAPK4 (MAPK p38delta) gene, and the alternatively spliced SAPK2 gene coding for CSaids binding protein CSBP2 and a MAPK p38beta LIKE protein. Contains ESTs, STSs and two predicted CpG islands, complete sequence.	Homo sapiens	34,490	23-Nov-99
rxa01954 963	GB_BA1:SC4H8	15560	AL020958	Streptomyces coelicolor cosmid 4H8.	Streptomyces coelicolor	37,960	10-DEC-1997
	GB_GSS3:B91274	183	B91274	CIT-HSP-2168G14.TF CIT-HSP Homo sapiens genomic clone 2168G14, genomic survey sequence.	Homo sapiens	36,066	25-Jun-98

TABLE 4: ALIGNMENT RESULTS

	GB_BA1:SC4H8	15560	AL020958	Streptomyces coelicolor cosmid 4H8.	Streptomyces coelicolor	39,457	10-DEC-1997
rxa01975 2019	GB_BA2:CGU13922	4412	U13922	Corynebacterium glutamicum putative type II 5-cytosoine methyltransferase (cglIM) and putative type II restriction endonuclease (cglIR) and putative type I or type III restriction endonuclease (clgIIR) genes, complete cds.	Corynebacterium glutamicum	99,950	3-Feb-98
	GB_BA1:SPSNBCDE	22449	Y11548	S.pristinaespiralis snbC gene & snbDE gene.	Streptomyces pristinaespiralis	36,657	25-Apr-97
	GB_BA1:SPSNBCGEN	22449	X98690	S.pristinaespiralis snbC and snbDE genes.	Streptomyces pristinaespiralis	36,657	24-MAR-1997
rxa01998 831	GB_BA2:AF121000	19751	AF121000	Corynebacterium glutamicum strain 22243 R-plasmid pAG1, complete sequence.	Corynebacterium glutamicum	40,520	14-Apr-99
	GB_BA2:AF121000	19751	AF121000	Corynebacterium glutamicum strain 22243 R-plasmid pAG1, complete sequence.	Corynebacterium glutamicum	54,699	14-Apr-99
	GB_BA1:FVBPOAD2A	45519	D26094	Flavobacterium sp. plasmid pOAD2 DNA, whole sequence.	Flavobacterium sp.	38,562	6-Feb-99
rxa02002 478	GB_BA1:STYPRFC	2140	D50496	Salmonella typhimurium gene for peptide release factor 3/RF3, complete cds.	Salmonella typhimurium	53,289	10-Feb-99
	GB_BA2:U32846	11650	U32846	Haemophilus influenzae Rd section 161 of 163 of the complete genome.	Haemophilus influenzae Rd	47,265	29-MAY-1998
	GB_BA2:AF072440	4316	AF072440	Enterobacter gergoviae GTPase (bipA) gene, partial cds; glutamine synthetase (glnA) and nitrogen regulatory protein (ntrB) genes, complete cds; and nitrogen regulatory protein (ntrC) gene, partial cds.	Enterobacter gergoviae	37,284	30-OCT-1998
rxa02015 619	GB_PL2:AF015560	2681	AF015560	Neurospora crassa RO11 (ro-11) gene, complete cds.	Neurospora crassa	38,953	3-Sep-97
	GB_GSS13:AQ497173	511	AQ497173	HS_5193_B2_A10_T7A RPCI-11 Human Male BAC Library Homo sapiens genomic clone Plate=769 Col=20 Row=B, genomic survey sequence.	Homo sapiens	37,086	28-Apr-99
	GB_PL1:SPAC27D7	35892	AL009227	S.pombe chromosome I cosmid c27D7.	Schizosaccharomyces pombe	39,016	25-MAR-1999
rxa02025 774	GB_BA1:ECOUW93	338534	U14003	Escherichia coli K-12 chromosomal region from 92.8 to 00.1 minutes.	Escherichia coli	39,108	17-Apr-96
	GB_BA2:AE000493	10819	AE000493	Escherichia coli K-12 MG1655 section 383 of 400 of the complete genome.	Escherichia coli	39,108	12-Nov-98
	GB_BA1:ECOPMSR	1270	M89992	Escherichia coli peptide methionine sulfoxide reductase gene, complete cds.	Escherichia coli	50,329	26-Apr-93
rxa02065 771	GB_BA2:MSU87307	1520	U87307	Mycobacterium smegmatis extracytoplasmic function alternative sigma factor (sigE) gene, complete cds.	Mycobacterium smegmatis	59,533	07-MAY-1997
	GB_BA1:MTCI61	13540	Z98260	Mycobacterium tuberculosis H37Rv complete genome; segment 53/162.	Mycobacterium tuberculosis	57,833	17-Jun-98
	GB_BA2:MTU87242	3690	U87242	Mycobacterium tuberculosis sigma factor SigE (sigE) and HtrA (htrA) genes, complete cds.	Mycobacterium tuberculosis	57,833	08-MAY-1997
	GB_BA1:MTCY338	29372	Z74697	Mycobacterium tuberculosis H37Rv complete genome; segment 127/162.	Mycobacterium tuberculosis	38,050	17-Jun-98
rxa02078 981	GB_BA1:MLCB1243	42926	AL023635	Mycobacterium leprae cosmid B1243.	Mycobacterium leprae	53,733	27-Aug-99
	GB_BA1:MSGB1723CS	38477	L78825	Mycobacterium leprae cosmid B1723 DNA sequence.	Mycobacterium leprae	53,733	15-Jun-96
rxa02110 741	GB_EST20:AA894760	281	AA894760	oj55a09.s1 NCI_CGAP_Kid3 Homo sapiens cDNA clone IMAGE:1502200 3', mRNA sequence.	Homo sapiens	39,928	9-Jun-98
	GB_EST38:AL119293	323	AL119293	DKFZp761B161_r1 761 (synonym: hamy2) Homo sapiens cDNA clone DKFZp761B161 5', mRNA sequence.	Homo sapiens	34,579	27-Sep-99
	GB_PR3:HSJ1031J8	155213	AL118523	Human DNA sequence from clone RP5-1031J8 on chromosome 20, complete sequence.	Homo sapiens	32,341	03-DEC-1999
rxa02167 1383	GB_BA1:MTCI125	37432	Z98268	Mycobacterium tuberculosis H37Rv complete genome; segment 76/162.	Mycobacterium tuberculosis	63,215	17-Jun-98
	GB_BA1:MLCB1351	38936	Z95117	Mycobacterium leprae cosmid B1351.	Mycobacterium leprae	38,240	24-Jun-97
	GB_BA1:U00021	39193	U00021	Mycobacterium leprae cosmid L247.	Mycobacterium leprae	37,964	29-Sep-94
rxa02174 477	GB_BA1:CGGLTG	3013	X66112	C.glutamicum glt gene for citrate synthase and ORF.	Corynebacterium glutamicum	100,000	17-Feb-95

TABLE 4: ALIGNMENT RESULTS

	GB_PR4:AF117829	320250	AF117829	Homo sapiens 8q21.3: RICK gene, complete sequence.	Homo sapiens	37,528	13-Jan-99
	GB_PR4:AF117829	320250	AF117829	Homo sapiens 8q21.3: RICK gene, complete sequence.	Homo sapiens	40,733	13-Jan-99
rxa02182							
rxa02204 1383	GB_BA1:MTCY261	27322	Z97559	Mycobacterium tuberculosis H37Rv complete genome; segment 95/162.	Mycobacterium tuberculosis	39,846	17-Jun-98
	GB_BA1:ECU82664	139818	U82664	Escherichia coli minutes 9 to 11 genomic sequence.	Escherichia coli	47,528	11-Jan-97
	GB_BA2:AE000158	10143	AE000158	Escherichia coli K-12 MG1655 section 48 of 400 of the complete genome.	Escherichia coli	47,528	12-Nov-98
rxa02228 1026	GB_HTG2:AC007962	172091	AC007962	Homo sapiens chromosome 17 clone 2511_J_5 map 17, *** SEQUENCING IN PROGRESS ***, 25 unordered pieces.	Homo sapiens	39,051	3-Jul-99
	GB_HTG2:AC007962	172091	AC007962	Homo sapiens chromosome 17 clone 2511_J_5 map 17, *** SEQUENCING IN PROGRESS ***, 25 unordered pieces.	Homo sapiens	39,051	3-Jul-99
	GB_HTG3:AC008363	131230	AC008363	Drosophila melanogaster chromosome 3 clone BACR14H24 (D989) RPCI-98 14.H.24 map 92A-92A strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 91 unordered pieces.	Drosophila melanogaster	31,957	3-Aug-99
rxa02236 441	GB_BA2:MSU75344	1458	U75344	Mycobacterium smegmatis integration host factor (mIHF) gene, complete cds.	Mycobacterium smegmatis	63,908	4-Aug-98
	GB_BA1:MTCY21B4	39150	Z80108	Mycobacterium tuberculosis H37Rv complete genome; segment 62/162.	Mycobacterium tuberculosis	58,957	23-Jun-98
	GB_BA2:AF077324	5228	AF077324	Rhodococcus equi strain 103 plasmid RE-VP1 fragment f.	Rhodococcus equi	40,639	5-Nov-98
rxa02242 630	GB_EST30:AI667039	548	AI667039	fc24h04.y1 Zebrafish WashU MPIMG EST Danio rerio cDNA 5' similar to TR:O93510 O93510 HOMOGENIN. ;, mRNA sequence.	Danio rerio	46,903	18-MAY-1999
	GB_EST30:AI667039	548	AI667039	fc24h04.y1 Zebrafish WashU MPIMG EST Danio rerio cDNA 5' similar to TR:O93510 O93510 HOMOGENIN. ;, mRNA sequence.	Danio rerio	38,445	18-MAY-1999
rxa02243 1068	GB_EST8:AA050680	515	AA050680	mj20f12.r1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:476687 5', mRNA sequence.	Mus musculus	40,313	9-Sep-96
	GB_EST28:AI509997	372	AI509997	mj20f12.y1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:476687 5', mRNA sequence.	Mus musculus	40,431	12-MAR-1999
	GB_EST27:AI426148	445	AI426148	mj20f12.x1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE:476687 3', mRNA sequence.	Mus musculus	45,775	09-MAR-1999
rxa02252 1544	GB_BA1:MTCY21B4	39150	Z80108	Mycobacterium tuberculosis H37Rv complete genome; segment 62/162.	Mycobacterium tuberculosis	63,017	23-Jun-98
	GB_PAT:I32742	5589	I32742	Sequence 1 from patent US 5589355.	Unknown.	66,077	6-Feb-97
	EM_BA1:AB003693	5589	AB003693	Corynebacterium ammoniagenes DNA for rib operon, complete cds.	Corynebacterium ammoniagenes	66,077	03-OCT-1997 (Rel. 52, Created)
rxa02260 354	GB_BA1:CORPEPC	4885	M25819	C.glutamicum phosphoenolpyruvate carboxylase gene, complete cds.	Corynebacterium glutamicum	100,000	15-DEC-1995
	GB_PAT:A09073	4885	A09073	C.glutamicum ppg gene for phosphoenol pyruvate carboxylase.	Corynebacterium glutamicum	100,000	25-Aug-93
	GB_BA1:CGL007732	4460	AJ007732	Corynebacterium glutamicum 3' ppc gene, secG gene, amt gene, ocd gene and 5' soxA gene.	Corynebacterium glutamicum	100,000	7-Jan-99
rxa02290 522	GB_GSS11:AQ262166	588	AQ262166	CITBI-E1-2509J2.TF CITBI-E1 Homo sapiens genomic clone 2509J2, genomic survey sequence.	Homo sapiens	41,505	24-OCT-1998
	GB_HTG5:AC006209	233854	AC006209	Homo sapiens clone RP11-546D14, *** SEQUENCING IN PROGRESS ***, 85 unordered pieces.	Homo sapiens	40,719	19-Nov-99

TABLE 4: ALIGNMENT RESULTS

	GB_VI:AF107100	2335	AF107100	Ecotropis obliqua nuclear polyhedrosis virus ecdysteroid UDP-glucosyltransferase gene, complete cds.	Ecotropis obliqua nuclear polyhedrosis virus	38,606	4-Apr-99
rxa02291 777	GB_PL1:ATF17M5	96475	AL035678	Arabidopsis thaliana DNA chromosome 4, BAC clone F17M5 (ESSA project).	Arabidopsis thaliana	35,195	11-MAR-1999
	GB_HTG4:AC007621	335275	AC007621	Homo sapiens chromosome 12p12-21.8-27.2 clone RPCI11-757G14, ***SEQUENCING IN PROGRESS ***, 142 unordered pieces.	Homo sapiens	36,471	21-OCT-1999
	GB_HTG4:AC007621	335275	AC007621	Homo sapiens chromosome 12p12-21.8-27.2 clone RPCI11-757G14, ***SEQUENCING IN PROGRESS ***, 142 unordered pieces.	Homo sapiens	36,471	21-OCT-1999
rxa02323 1047	GB_PL1:YSKERD2A	1248	M34844	K.lactis ER lumen protein retaining receptor (ERD2) gene, complete cds.	Kluyveromyces lactis	37,168	27-Apr-93
	GB_PL2:CNS01AFM	720	AL112874	Botrytis cinerea strain T4 cDNA library under conditions of nitrogen deprivation.	Botryotinia fuckeliana	39,638	2-Sep-99
	GB_PR1:HAAXTRSYV	6972	X90840	H.sapiens mRNA for axonal transporter of synaptic vesicles.	Homo sapiens	38,454	28-MAY-1996
rxa02386 582	GB_OV:AF131057	1875	AF131057	Gallus gallus substance P receptor (ASPR) mRNA, complete cds.	Gallus gallus	38,382	18-MAY-1999
	GB_HTG2:AC008225	110418	AC008225	Drosophila melanogaster chromosome 3 clone BACR03E11 (D818) RPCI-98 03.E.11 map 84C-84D strain y; cn bw sp, *** SEQUENCING IN PROGRESS***, 76 unordered pieces.	Drosophila melanogaster	39,236	2-Aug-99
	GB_EST10:AA142237	594	AA142237	CK00013.3prime CK Drosophila melanogaster embryo BlueScript Drosophila melanogaster cDNA clone CK00013 3prime, mRNA sequence.	Drosophila melanogaster	36,519	29-Nov-98
rxa02388 1785	GB_RO:RNY16563	12507	Y16563	Rattus norvegicus mRNA for brain-specific synapse-associated protein, Bassoon.	Rattus norvegicus	35,082	11-Aug-98
	GB_PR4:AF052224	15964	AF052224	Homo sapiens neuronal double zinc finger protein (ZNF231) mRNA, complete cds.	Homo sapiens	36,270	09-DEC-1998
	GB_PR1:AB007894	5650	AB007894	Homo sapiens KIAA0434 mRNA, partial cds.	Homo sapiens	36,970	13-Feb-99
rxa02413 615	GB_PR4:AC007102	176258	AC007102	Homo sapiens chromosome 4 clone C0162P16 map 4p16, complete sequence.	Homo sapiens	36,772	2-Jun-99
	GB_HTG3:AC011214	183414	AC011214	Homo sapiens clone 5_C_3, LOW-PASS SEQUENCE SAMPLING.	Homo sapiens	36,442	03-OCT-1999
	GB_HTG3:AC011214	183414	AC011214	Homo sapiens clone 5_C_3, LOW-PASS SEQUENCE SAMPLING.	Homo sapiens	36,442	03-OCT-1999
rxa02416 2952	GB_BA1:MSGB1133CS	42106	L78811	Mycobacterium leprae cosmid B1133 DNA sequence.	Mycobacterium leprae	65,083	15-Jun-96
	GB_BA1:MTCY06H11	38000	Z85982	Mycobacterium tuberculosis H37Rv complete genome; segment 73/162.	Mycobacterium tuberculosis	66,278	17-Jun-98
	GB_BA1:SCC54	30753	AL035591	Streptomyces coelicolor cosmid C54.	Streptomyces coelicolor	39,079	11-Jun-99
rxa02418 690	GB_BA1:MTCY06H11	38000	Z85982	Mycobacterium tuberculosis H37Rv complete genome; segment 73/162.	Mycobacterium tuberculosis	62,899	17-Jun-98
	GB_BA1:MSGB1133CS	42106	L78811	Mycobacterium leprae cosmid B1133 DNA sequence.	Mycobacterium leprae	66,473	15-Jun-96
	GB_BA1:SCI35	40909	AL031541	Streptomyces coelicolor cosmid I35.	Streptomyces coelicolor	35,958	9-Sep-98
rxa02429 2346	GB_BA1:MLCB1788	39228	AL008609	Mycobacterium leprae cosmid B1788.	Mycobacterium leprae	40,352	27-Aug-99
	GB_BA1:MTCY1A11	30850	Z78020	Mycobacterium tuberculosis H37Rv complete genome; segment 83/162.	Mycobacterium tuberculosis	57,417	17-Jun-98
	GB_PL2:AC007153	103223	AC007153	Arabidopsis thaliana chromosome I BAC F3F20 genomic sequence, complete sequence.	Arabidopsis thaliana	36,104	17-MAY-1999
rxa02436 684	GB_BA1:MTCY10H4	39160	Z80233	Mycobacterium tuberculosis H37Rv complete genome; segment 2/162.	Mycobacterium tuberculosis	63,274	17-Jun-98
	GB_BA1:MLCB1770	37821	Z70722	Mycobacterium leprae cosmid B1770.	Mycobacterium leprae	62,719	29-Aug-97
	GB_BA1:SCH69	35824	AL079308	Streptomyces coelicolor cosmid H69.	Streptomyces coelicolor	40,237	15-Jun-99
rxa02445 1812	GB_PR3:HS864I18	106018	AL031293	Human DNA sequence from clone 864I18 on chromosome 1p36.11-36.33. Contains ESTs, STSs, GSSs, genomic marker D1S2728 and a ca repeat polymorphism, complete sequence.	Homo sapiens	37,409	23-Nov-99

09602839 . 062300

TABLE 4: ALIGNMENT RESULTS

	GB_PR3:HS864I18	106018	AL031293	Human DNA sequence from clone 864I18 on chromosome 1p36.11-36.33. Contains ESTs, STSs, GSSs, genomic marker D1S2728 and a ca repeat polymorphism, complete sequence.	Homo sapiens	38,679	23-Nov-99
rxa02456 741	GB_BA2:AF144091	2900	AF144091	Mycobacterium smegmatis catechol 1,2-dioxygenase (catA) gene, partial cds; muconolactone isomerase (catC) and sigma factor SigH (sigH) genes, complete cds; and unknown genes.	Mycobacterium smegmatis	57,085	15-Jul-99
	GB_BA1:MTCY7D11	22070	Z95120	Mycobacterium tuberculosis H37Rv complete genome; segment 138/162.	Mycobacterium tuberculosis	35,534	17-Jun-98
	GB_STS:G36947	418	G36947	SHGC-56623 Human Homo sapiens STS cDNA, sequence tagged site.	Homo sapiens	36,591	1-Jan-98
rxa02462 1941	EM_PAT:E09053	2538	E09053	gDNA encoding secA protein.	Corynebacterium glutamicum	99,528	07-OCT-1997 (Rel. 52, Created)
	GB_BA1:MTY20B11	36330	Z95121	Mycobacterium tuberculosis H37Rv complete genome; segment 139/162.	Mycobacterium tuberculosis	38,632	17-Jun-98
	GB_BA2:MBU66080	4049	U66080	Mycobacterium bovis SecA (secA) gene, complete cds.	Mycobacterium bovis	68,353	3-Sep-98
rxa02476 1002	GB_BA1:AB009078	2686	AB009078	Brevibacterium saccharolyticum gene for L-2.3-butanediol dehydrogenase, complete cds.	Brevibacterium saccharolyticum	97,309	13-Feb-99
	GB_HTG2:AC007933	152224	AC007933	Homo sapiens chromosome 17 clone hRPC.908_O_12 map 17, ***SEQUENCING IN PROGRESS ***, 11 unordered pieces.	Homo sapiens	39,959	30-Jun-99
	GB_HTG2:AC007933	152224	AC007933	Homo sapiens chromosome 17 clone hRPC.908_O_12 map 17, ***SEQUENCING IN PROGRESS ***, 11 unordered pieces.	Homo sapiens	39,959	30-Jun-99
rxa02502 1515	GB_PR2:AP000548	128077	AP000548	Homo sapiens genomic DNA, chromosome 22q11.2, Cat Eye Syndrome region, clone:KB556G11.	Homo sapiens	36,965	01-OCT-1999
	GB_BA1:MSGY348	40056	AD000020	Mycobacterium tuberculosis sequence from clone y348.	Mycobacterium tuberculosis	38,198	10-DEC-1996
	GB_PR2:AP000548	128077	AP000548	Homo sapiens genomic DNA, chromosome 22q11.2, Cat Eye Syndrome region, clone:KB556G11.	Homo sapiens	35,839	01-OCT-1999
	GB_BA1:MTCY1A10	25949	Z95387	Mycobacterium tuberculosis H37Rv complete genome; segment 117/162.	Mycobacterium tuberculosis	38,806	17-Jun-98
rxa02509 1994	GB_BA1:MLCL581	36225	Z96801	Mycobacterium leprae cosmid L581.	Mycobacterium leprae	38,532	24-Jun-97
	GB_BA1:MTCY1A10	25949	Z95387	Mycobacterium tuberculosis H37Rv complete genome; segment 117/162.	Mycobacterium tuberculosis	39,036	17-Jun-98
rxa02523 942	GB_BA1:MLCB250	40603	Z97369	Mycobacterium leprae cosmid B250.	Mycobacterium leprae	47,284	27-Aug-99
	GB_EST25:AU041363	542	AU041363	AU041363 Mouse four-cell-embryo cDNA Mus musculus cDNA clone J1001B09 3', mRNA sequence.	Mus musculus	39,180	04-DEC-1998
	GB_EST9:C22241	332	C22241	C22241 Miyagawa-wase satsuma mandarin orange (M.Omura) Citrus unshiu cDNA clone pcMFRI802.43, mRNA sequence.	Citrus unshiu	42,638	29-Jun-98
rxa02557 711	GB_HTG3:AC010964	41594	AC010964	Homo sapiens chromosome 17 clone 3023_F_18 map 17, *** SEQUENCING IN PROGRESS ***, 3 unordered pieces.	Homo sapiens	36,234	28-Sep-99
	GB_HTG3:AC010964	41594	AC010964	Homo sapiens chromosome 17 clone 3023_F_18 map 17, *** SEQUENCING IN PROGRESS ***, 3 unordered pieces.	Homo sapiens	36,234	28-Sep-99
	GB_PR2:AC000003	122228	AC000003	Homo sapiens chromosome 17, clone 104H12, complete sequence.	Homo sapiens	36,222	07-OCT-1997
rxa02563 855	GB_GSS14:AQ570921	491	AQ570921	HS_5356_B1_H12_T7A RPCI-11 Human Male BAC Library Homo sapiens genomic clone Plate=932 Col=23 Row=P, genomic survey sequence.	Homo sapiens	35,191	1-Jun-99
	GB_EST27:AI425057	501	AI425057	tg50g05.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2112248 3', mRNA sequence.	Homo sapiens	38,723	30-MAR-1999

TABLE 4: ALIGNMENT RESULTS

	GB_EST6:N63837	469	N63837	za26h12.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:293735 3', mRNA sequence.	Homo sapiens	36,725	01-MAR-1996
rxa02590 1059	GB_PAT:I92041	858	I92041	Sequence 8 from patent US 5726299.	Unknown.	34,837	01-DEC-1998
	GB_PAT:I78752	858	I78752	Sequence 8 from patent US 5693781.	Unknown.	34,837	3-Apr-98
	GB_HTG2:AC006936	221373	AC006936	Drosophila melanogaster chromosome 3 clone BACR48I01 (D484) RPCI-98 48.I.1 map 93E4-93E7 strain y; cn bw sp, *** SEQUENCING IN PROGRESS ***, 63 unordered pieces.	Drosophila melanogaster	36,103	2-Aug-99
rxa02608 2094	GB_BA1:CGCOP1G	2547	X66078	C.glutamicum cop1 gene for PS1.	Corynebacterium glutamicum	99,140	30-Jun-93
	GB_PAT:A26027	2547	A26027	C.melassecola gene for extracellular antigen PS1.	Corynebacterium melassecola	99,045	2-Apr-95
	GB_HTG6:AC008180_2	110000	AC008180	Homo sapiens clone RP11-292L5, *** SEQUENCING IN PROGRESS ***, 152 unordered pieces.	Homo sapiens	35,990	AC008180
rxa02625 886	GB_BA1:MTV012	70287	AL021287	Mycobacterium tuberculosis H37Rv complete genome; segment 132/162.	Mycobacterium tuberculosis	39,135	23-Jun-99
	GB_BA1:SC8D9	38681	AL035569	Streptomyces coelicolor cosmid 8D9.	Streptomyces coelicolor	65,537	26-Feb-99
	GB_BA1:MLCB637	44882	Z99263	Mycobacterium leprae cosmid B637.	Mycobacterium leprae	63,995	17-Sep-97
rxa02671 702	GB_EST38:AW029724	634	AW029724	EST272979 tomato callus, TAMU Lycopersicon esculentum cDNA clone cLEC28I17 similar to beta-ketoacyl-ACP synthase, putative, mRNA sequence.	Lycopersicon esculentum	34,750	15-Sep-99
	GB_GSS6:AQ843663	631	AQ843663	nbxb0024L12r CUGI Rice BAC Library Oryza sativa genomic clone nbxb0024L12r, genomic survey sequence.	Oryza sativa	41,971	04-OCT-1999
	GB_EST38:AW029724	634	AW029724	EST272979 tomato callus, TAMU Lycopersicon esculentum cDNA clone cLEC28I17 similar to beta-ketoacyl-ACP synthase, putative, mRNA sequence.	Lycopersicon esculentum	38,760	15-Sep-99
rxa02686 1260	GB_BA1:CORPHEA	1088	M13774	C.glutamicum pheA gene encoding prephenate dehydratase, complete cds.	Corynebacterium glutamicum	44,279	26-Apr-93
	GB_PAT:E06110	948	E06110	DNA encoding prephenate dehydratase.	Corynebacterium glutamicum	43,836	29-Sep-97
	GB_PAT:E04484	948	E04484	DNA encoding prephenate dehydratase.	Corynebacterium glutamicum	43,836	29-Sep-97
rxa02692 1389	GB_BA1:MTCY1A6	37751	Z83864	Mycobacterium tuberculosis H37Rv complete genome; segment 159/162.	Mycobacterium tuberculosis	35,699	17-Jun-98
	GB_PAT:I60487	1260	I60487	Sequence 3 from patent US 5656470.	Unknown.	67,383	07-OCT-1997
	GB_BA1:MSGY409	41321	AD000017	Mycobacterium tuberculosis sequence from clone y409.	Mycobacterium tuberculosis	63,413	10-DEC-1996
rxa02726 3057	GB_BA1:MTCY48	35377	Z74020	Mycobacterium tuberculosis H37Rv complete genome; segment 69/162.	Mycobacterium tuberculosis	65,390	17-Jun-98
	GB_PAT:AR009609	3905	AR009609	Sequence 1 from patent US 5756327.	Unknown.	65,160	04-DEC-1998
	GB_PAT:AR009610	1487	AR009610	Sequence 3 from patent US 5756327.	Unknown.	63,792	04-DEC-1998
rxa02731 2220	GB_BA1:MTCY01B2	35938	Z95554	Mycobacterium tuberculosis H37Rv complete genome; segment 72/162.	Mycobacterium tuberculosis	70,069	17-Jun-98
	GB_BA1:MSGB1133CS	42106	L78811	Mycobacterium leprae cosmid B1133 DNA sequence.	Mycobacterium leprae	69,559	15-Jun-96
	GB_BA1:MLUVRB	2286	X12578	Micrococcus luteus gene homologous to E.coli uvrB gene.	Micrococcus luteus	63,361	12-Sep-93
rxa02742 2472	GB_GSS12:AQ364217	467	AQ364217	nbxb0060L21f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0060L21f, genomic survey sequence.	Oryza sativa	37,337	3-Feb-99
	GB_GSS12:AQ364217	467	AQ364217	nbxb0060L21f CUGI Rice BAC Library Oryza sativa genomic clone nbxb0060L21f, genomic survey sequence.	Oryza sativa	39,123	3-Feb-99
rxa02748 1764	GB_BA1:CAJ10319	5368	AJ010319	Corynebacterium glutamicum amtP, glnB, glnD genes and partial ftsY and srp genes.	Corynebacterium glutamicum	99,888	14-MAY-1999
	GB_BA1:MTCY338	29372	Z74697	Mycobacterium tuberculosis H37Rv complete genome; segment 127/162.	Mycobacterium tuberculosis	38,016	17-Jun-98

09602839 .062300

TABLE 4: ALIGNMENT RESULTS

		GB_BA1:MSGB32CS	36404	L78818	Mycobacterium leprae cosmid B32 DNA sequence.	Mycobacterium leprae	62,730	15-Jun-96
		GB_BA1:MTCYW318	2803	Z97051	Mycobacterium tuberculosis H37Rv complete genome; segment 112/162.	Mycobacterium tuberculosis	39,294	17-Jun-98
rxa02788	2787	GB_BA1:MSGB937CS	38914	L78820	Mycobacterium leprae cosmid B937 DNA sequence.	Mycobacterium leprae	60,729	15-Jun-96
		GB_BA1:MLCB1259	38807	AL023591	Mycobacterium leprae cosmid B1259.	Mycobacterium leprae	66,993	27-Aug-99
					Paracoccus denitrificans phosphate acetyltransferase (pta) gene, complete cds,			
rxa02837	274	GB_BA2:PDU08864	2215	U08864	and insertion sequence IS1248a, complete sequence.	Paracoccus denitrificans	73,723	30-Nov-95
					Paracoccus denitrificans insertion sequence IS1248b, complete sequence.			
		GB_BA1:PDU08856	1393	U08856	Zymomonas mobilis genomic DNA clone encoding ORF1 to 4.	Paracoccus denitrificans	73,723	30-Nov-95
		GB_BA1:ZMO009974	4494	AJ009974	B.lactofermentum orf1 gene and sigB gene.	Zymomonas mobilis	37,500	3-Aug-99
rxs03207	1123	GB_BA1:BLSIGBGN	2906	Z49824	Mycobacterium tuberculosis H37Rv complete genome; segment 120/162.	Corynebacterium glutamicum	99,555	25-Apr-96
		GB_BA1:MTCY05A6	38631	Z96072	Mycobacterium tuberculosis H37Rv sigma factor MysA (mysA) and sigma factor	Mycobacterium tuberculosis	65,474	17-Jun-98
		GB_BA1:MTU10059	5900	U10059	MysB (mysB) genes, complete cds.	Mycobacterium tuberculosis	65,474	30-Jan-96